A DESCRIPTIVE STUDY OF PHENOTYPIC VARIATIONS AND GENOTYPE – PHENOTYPE CORRELATION IN A COHORT OF SRI LANKAN PATIENTS WITH THE TURNER SYNDROME PHENOTYPE

BY

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ABSTRACT

Background

Turner syndrome (TS) is the most common sex chromosomal abnormality seen in females with the occurrence of 1 in 2500 – 3000 live female births. It is characterized by the absence of all or part of a second sex chromosome involving the terminal end of its short arm which results in a variety of clinical features. Major clinical features of TS are short stature and primary amenorrhoea while features such as short and/or webbed neck, wide carrying angle and shield shaped chest are minor manifestations. There are different karyotpe abnormalities which cause TS. 45,X, 46,XX/45,X and 46,X, i(X)(q10) are the commonest. Different karyotype abnormalities may cause different phenotypic features. In this study the main objective was to find out the cytogenetic abnormalities in patients with Turner syndrome in Sri Lanka, phenotypic features and the correlation between the cytogenetic abnormalities and the phenotypic features in patients with Turner syndrome in Sri Lanka. The second objective of the study was to detect mutations in 4 exons out of 15 exons of the *PTPN11* (Protein Tyrosine Phosphatase Non receptor type 11) gene which is a common gene responsible for Noonan syndrome (NS) in Sri Lankan patients who had a clinical diagnosis of TS and whose karyotype was found to be normal (46,XX). Noonan syndrome is a major differential diagnosis of Turner syndrome as both syndromes share similar phenotypic features.

Method

In order to assess patients with Turner syndrome, 33 patients who had an abnormal karyotype causing TS were interviewed and examined. Twenty four patients with a clinical phenotype of TS, whose karyotype was normal, were screened for mutations in the exon 3, 8, 9 and 13 of the *PTPN11* gene.

Results

The cytogenetic abnormalities were, 13 (39.4%) - 45,X karyotype, 11 (33.3%) - 46, XX/45, X karyotype, 5 (15.2%) - 46,X, i(X)(q10) karyotype, 3 (9.1%) - 45,X/46,X, i(X) (q10) karyotype and 1 (3%) - X/autosome translocation. The clinical manifestations were more severe in the first category than the other categories. Features such as primary amenorrhoea, ptosis, low posterior hair line, shield shaped chest and widely spaced nipples showed a significant association with the cytogenetic abnormalities. No mutations were found in exons (3, 8, 9 and 13) of the *PTPN11* gene in patients who had a normal karyotype.

Conclusion

In patients with TS features and an abnormal karyotype the clinical features correlated with the karyotype. In patients who had a normal karyotype, three sequence variations were found in exon 3, 9 and IVS9 (intervening sequence of exon 9 and 10) of *PTPN11* gene, out of which two needs further investigations to determine the functional significance.

1. INTRODUCTION

1.1 Background of Turner Syndrome

Turner syndrome (TS) is the most common sex chromosome abnormality seen in females. It is a disorder in females characterized by the absence of all or part of a second sex chromosome involving the terminal end of its short arm which results in a variety of clinical findings (Sybert *et al.* 2004; Davenport 2010). TS occur in one out of 2,500 to 3,000 live female births. The 45, X karyotype (Monosomy X) accounts for 3% of all conceptions but almost 99% of these cases end up in spontaneous abortions. It is said that out of first trimester abortions 10 % are due to TS. Maternal age is not a risk factor for TS as it is in Down syndrome and also there are no clearly known risk factors. Recurrence in future pregnancies is very rare (Morgan 2007).

1.2 Cytogenetic variation of TS

Approximately half of the TS patients have 45, X karyotype (Turner monosomy/ classical Turner). Chromosome mosaicism (45, X/46, XX) is found in twenty to thirty percent of TS patients while the rest arise from structural abnormalities with one normal X and a structurally rearranged second X chromosome. These structural abnormalities include deletions, duplications, inversions, translocations, isochromosomes and ring chromosomes which are associated with chromosomal breaks and significant imbalance of gene content of the X chromosome. However, such abnormalities are generally well tolerated because of the preferential inactivation of the abnormal X chromosome (Leppig *et al.* 2001).

Isochromosome (isoXq) is one of the rearrangements which can occur structurally and it consists of duplications of the long arm (q) of the X chromosome with the concurrent loss of the short arm (p). There are patients with non mosaic isochromosomes of the chromosome X long arm [46,X,i(X)(q10)] and mosaicism of monosomy X and isochromosome Xq both (Cetin 2011). Ring formation (rX) is another variant of TS which occurs due to structural

rearrangement of the second X chromosome and deletions of the short and long arms of the X chromosome (Xp- and Xq-) (Oliveira 2011).

Other uncommon cytogenetic variation of TS is X/autosome translocations and is associated with a variable phenotype including features of TS (Sills *et al.*).

1.3 X inactivation

In mammals, females have two X chromosomes which are rich in genes (1000) and males have only one X chromosome and a Y chromosome which contains only few genes (200). For the dosage compensation of genes, a phenomenon called X inactivation occurs in cells in mammals and this is initiated by X inactivation specific transcript (*XIST*) gene which is transcribed only by the inactive X cells. One X chromosome is randomly inactivated during the first week of life, when there are fewer than 200 embryonic cells. This inactivation does not occur in all the genes in the second X chromosome. The genes in the pseudo autosomal regions (the regions in the X chromosomes which are homologous with the Y chromosome) escape inactivation (Gartler 2001). The loss of these genes which escaped inactivation are responsible for the phenotypic manifestations characteristic of Turner syndrome, such as short stature (Morgan 2007). In most patients with structural abnormalities of the X chromosome, this beneficial effect of X inactivation results in a mild phenotype similar to that found in TS patients with the classical 45, X karyotype. However, in cases of ring X chromosomes and X/autosome translocations, the incidence of mental retardation and other congenital abnormalities has been shown to be significantly higher than in monosomy X TS due to failed or partial X inactivation (Davenport ; Leppig *et al.* 2001). In normal females, most genes on one X chromosome are silenced during X inactivation but it has been observed that approximately 20% of genes on the inactivated X chromosome escape inactivation (Carrel *et al.* 1999). Despite normal females having a single functioning X chromosome with the other X chromosome inactivated through *XIST* gene, the phenotype of normal females

differs significantly from that of TS patients. The reason being that the genes which escape inactivation during embryogenesis are thought to play an important role in the development of normal female phenotype (Carrel *et al.* 2005).

1.4 Pathogenesis of TS

Majority of cases with TS (60%) arise due to non-dysjunction during paternal meiosis but no risk factors have so far been identified for this chromosomal loss. Increased maternal age has not been shown to be a risk factor (Denniston *et al.* 2004). Although in 80% of patients, the X chromosome is from the mother, imprinting has no place in its pathophysiology, as the phenotype is not determined by the maternal or paternal origin of the X chromosome. Genetic abnormalities in TS are due to the absence of genes on the X chromosome i.e. the phenotypic features of TS can be attributed to a haploinsufficiency of genes of the X chromosome which escapes inactivation. For example, in patients with TS and deletions at the end of the short arm of the X chromosome (Xp-) involving haploinsufficiency of the *SHOX* (short stature homeobox) gene, short stature, skeletal abnormalities like cubitus valgus, Madelung deformity and high arched palate can be seen (Oliveira 2011).

1.5 Clinical Features of Classical TS

TS may affect many body systems including lymphatic, cardiac, renal, orthopedic, ocular, auditory, skin, ovarian development/reproduction, growth and psychosocial development. TS patients have a varied phenotypic pattern. The presentation varies throughout a patient's life (Morgan 2007). Dorsal oedema of hands and feet, low birth weight and reduced length are the prominent features seen in neonates with TS. Phenotypic features in children and adults include short stature, neck webbing, low posterior hairline, broad chest with apparent widely spaced nipples, cubitus valgus (wide carrying angle) and hyper convex fingernails. The facial features include small mandible, prominent ears, epicanthic folds and high arched palate. The

cardiovascular features include bicuspid aortic valves, aortic coarctation, aortic stenosis, mitral valve prolapse, anomalous pulmonary venous drainage and hypertension. Renal anomalies include pelvic and horseshoe kidneys, double collecting system and renal agenesis. Primary amenorrhoea, infertility and streak ovaries are commonly present. Thyroid dysfunction, diabetes mellitus type II, osteoporosis, dyslipidaemia and liver function abnormalities are some of the metabolic derangements present in patients with TS. Neurological disorders such as sensorineural deafness, delayed motor development and delayed language skills can also be seen in these patients. Psychological disorders include dependence, delayed emotional maturity and negative body image (Denniston *et al.* 2004). Though the overall IQ (Intelligence Quotient) is normal there are prominent cognitive weaknesses in executive and visuospatial functions (Marzelli 2011). There are ocular manifestations associated with TS like amblyopia, strabismus, hypermetropia, ptosis and bilateral epicanthus (Adhikary 1981). Brunnerova *et al.* reported the occurrence of strabismus and ptosis were higher in TS than in the average population (Brunnerova *et al.* 2007).

Mortality in TS in the neonatal period is mainly due to hypoplastic left heart and coarctation of the aorta and in adulthood because of cardiovascular disease, particularly aortic dissection. Obesity, with associated diabetes mellitus and hypertension, can also contribute to early mortality. Limited epidemiologic studies suggest that life expectancy is reduced by about 10 years (Natowicz *et al.* 1987).

1.6 Clinical features of TS variants

Patients with ring X chromosome show a more severe clinical phenotype including mental retardation, limb abnormalities and dysmorphic features (Dennis *et al.* 1993). However they do not show the “classic” TS features such as webbed neck, congenital lymphoedema, anteverted ears and epicanthic folds. These features result from in utero lymphoedema which normally occurs by 8 weeks’ gestation (Collins *et al.* 1994). It is said that the ring X

chromosome of the mother was most likely transmitted to the newborn. Therefore an ovum with the ring X chromosome can be fertile and can produce a viable zygote (Uehara 1997).

Isochromosome of the long arm of X chromosome (iXq) is the commonest structural aberration found in patients with TS. The definition of an isochromosome is a single functional centromere separating two arms which are mirror images of one another. It has also been used to designate a broader group of chromosome rearrangements which includes isodicentrics and duplications of genetically nonidentical arms (Lorda-Sanchez *et al.* 1991). Kerdanet *et al* confirmed that patients with X isochromosome TS have an increased risk of developing thyroiditis than the other TS patients (de Kerdanet *et al.* 1994).

Patients with X-autosome translocations show features of TS when the breakpoint is in the critical region of Xq and especially when the genes which normally escape X inactivation are affected (Crocker 1992).

In addition it is reported that hypothyroidism is more common in patients with mosaic form of TS [45,X/46,XX and 45,X/46,Xi(Xq)] (Nazarabadi 2005).

1.7 Genotype - phenotype correlation

Genotype - phenotype correlation in patients with TS has shown that specific regions of the X chromosome are responsible for certain clinical features. *SHOX* gene located on the short arm of X chromosome was one of the earliest discovered regions responsible for the short stature and skeletal abnormalities of TS (Belin *et al.* 1998). Recently it was found that mutations of the short stature homeobox gene (*SHOX*), was associated with the short stature phenotype in patients with TS and most patients with Léri-Weill dyschondrosteosis (Rappold 2002). The *SHOX* gene is located in the pseudoautosomal region 1 (PAR1), which is a 170 kb DNA segment, about 500 kb distant from telomeres and located at the end of the short arm of the X and Y chromosomes (Xp22.3andYp11.3) (Oliveira 2011).

Micrognathia, cubitus valgus, high-arched palate and short metacarpals and metatarsals are the main skeletal abnormalities seen in TS which are related to *SHOX* deletions. Madelung deformity which is characterized by shortening and bowing of the radius and dorsal dislocation of the distal ulna is only present in 8% of patients. Therefore it is possible to say, that patients with short stature, normal karyotype and one or several skeletal abnormalities associated with TS can be carriers of the *SHOX* gene mutations. So far, *SHOX* expression has not been detected in cardiac, renal or vascular organogenesis. It suggests that *SHOX* gene probably has no role in the development of nonskeletal somatic features in the TS phenotype. Haploinsufficiency of the *SHOX* gene also does not cause other clinical manifestations of TS such as ovarian failure and lymphoedema. The cause for higher frequency of middle ear infections in patients with *SHOX* gene deficiency could be due to skeletal abnormalities of the outer ear (Oliveira 2011). Morton described the genes located on the X chromosome which are responsible for nonspecific mental retardation (*MRX1, MRX2, MRX3)* (Mental retardation X-linked Type 1, 2, 3) (Morton 1992). The correlation between TS phenotype and the other X chromosome genes has been described by Davenport (Davenport 2010). In that study he reported that low posterior hair line, lymphoedema, nail dysplasia and webbed neck are caused due to haploinsufficiency of putative lymphatic gene which is located on the X chromosome. Similarly lack of one germ cell survival gene on the X chromosome is responsible for infertility, gonadal failure and delayed puberty. There are also several unknown genes on the X chromosome, in the absence of which other phenotypes such as renal malformations, hypertension, coarctation of aorta, bicuspid aortic valves, increased liver enzymes, hypothyroidism and diabetes mellitus can develop (Davenport 2010).

1.8 Noonan syndrome

Noonan syndrome (NS) is the main differential diagnosis for TS in the absence of karyotypic abnormality. It is a common autosomal dominant disease among children. It has an

inheritance with a predominance of maternal transmission. The incidence is estimated to be between 1:1000 and 1:2500 live births (Burgt 2007). Several genes have been associated with Noonan syndrome: *PTPN11, KRAS* (v-ki-ras2 Kirsten rat sarcoma viral oncogene homolog.), *SOS1* [Son of sevenless homolog1 (Ddrosophila)], *RAF1* (v-rat-1 murine leukemia viral oncogene homolog1), *NRAS* [neuroblastoma RAS viral (v-ras) oncogene homolog] and *SHOC2* (SOC-2 suppressor of clear homolog). Molecular genetic testing of the four most common Noonan syndrome genes is available and mutations have been identified in *PTPN11* in about 50%, *KRAS* in about <5%, *SOS1* in about 15% and *RAF* in 3 – 17%. Genotype – phenotype correlations have been reported. Short stature, which is the universal feature of TS, is also a feature of NS due to mutations in *PTPN11* gene (Yoshida *et al.* 2004).

1.8.1 Clinical Features of NS

Noonan syndrome affects multiple systems but is often under diagnosed due to its variable features and characteristic clinical symptoms which are similar to TS (van der Burgt 2007).

The facial appearance of NS shows considerable change with age, being most striking in the newborn period and middle childhood, and most subtle in the adult. In the neonates facial features include, tall forehead, hypertelorism with downslanting palpebral fissures, low-set, posteriorly rotated ears with a thickened helix, a deeply grooved philtrum with high, wide peaks to the vermillion border of the upper lip, and a short neck with excess nuchal skin and low posterior hairline. In infancy the main distinctive features are prominent eyes with horizontal fissures, hypertelorism, and thickened eye lids. The nose is depressed at the root, while the tip is bulbous. By childhood a myopathic appearance emerges with a lack of expression and in adolescence a facial shape of an inverted triangle is found with eyes less prominent and lengthening of the neck. By adulthood the skin appears transparent and wrinkle (Romano *et al.*).

Reduced adult height is a feature in Noonan syndrome with 50 – 70 % of individuals achieving the lower limit of normal. Many affected individuals have a normal length at birth but achieve a short adult height, due to abnormal growth hormone levels (Otten *et al.* 2009). Skeletal malformations are a feature of Noonan syndrome with pectus carinatum, pectus excavatum and scoliosis of the spine. Congenital heart disease occurs in 50%-80% of individuals. Pulmonary valve stenosis, often with dysplasia, is the most common heart defect and is found in 20%-50% of individuals. Hypertrophic cardiomyopathy, found in 20%-30% of individuals, may be present at birth or develop in infancy or childhood. Other structural defects include atrial and ventricular septal defects, branch pulmonary artery stenosis, and tetralogy of Fallot (Noonan 2006).

Coagulation screens such as prothrombin time, activated partial thromboplastin time, platelet count, and bleeding time may show abnormalities. Specific testing should identify the particular coagulation defect, such as von Willebrand disease, thrombocytopenia, varied coagulation factor defects (factors V, VIII, XI, XII, protein C), and platelet dysfunction. Presentation may be with nose bleeds, bruising or prolonged bleeding following injury or surgery. Menorragia may be present in females with Noonan syndrome (Derbent *et al.* 2010).

Adolescent males with Noonan syndrome typically experience delayed puberty. Affected individuals go through puberty starting at age 13 or 14 and have a reduced pubertal growth spurt. Most males with Noonan syndrome have cryptorchidism, which may be related to delayed puberty or to infertility later in life (Jorge *et al.* 2009). Females with Noonan syndrome typically have normal or delayed puberty and fertility will not be affected usually (van der Burgt 2007).

Noonan syndrome can cause a variety of other signs and symptoms. Most children diagnosed with Noonan syndrome have normal intelligence, but a small percentage has special

educational needs (10-15%), and some have intellectual disability (25%). Articulation deficiency is common (72%) but usually responds well to speech therapy. Language delay may be related to hearing loss, perceptual motor disabilities, or articulation deficiencies. Infants with Noonan syndrome may be born with puffy hands and feet caused by lymphoedema. Older individuals can also develop lymphedema, usually in the ankles and lower legs (van der Burgt 2007).

1.8.2 Etiology of NS

NS may occur sporadically and also inherited in an autosomal dominant pattern with a predominance of maternal transmission (van der Burgt 2007).

The genetic basis of NS is said to be the heterozygous gain-of-function mutations in various genes encoding proteins of the Ras-MAPK (Mitogen-activated protein kinase) signaling cascade (Zenker 2007).

It is said that missense mutations in the *PTPN11* gene on chromosome 12 (12q24) account for approximately 50% of NS cases. This is an enzyme which involved in a wide variety of intracellular signalcascades down-stream to receptors for growth factors, cytokines, and hormones and is required in several developmental processes. The configuration of SHP-2 is described as two src homology 2 domains, N-SH2 and C-SH2, at the amino terminus, a single central phosphatase domain (PTP) and a carboxy-terminal tail. Functionally, the N-SH2 and PTP interdomain interaction controls the switching of the SHP-2 protein between its active and inactive state. The majority of mutations associated with NS affect such interaction destabilizing the catalytically inactive conformation of the protein. This results in a gain of function of SHP-2. It is reported that there is a clustering of mutations within the N-SH2 domain (exon 3) and PTP-domain (exons 7, 8, and 13), with the c.922 A>G substitution (Asn308Asp) being the most common mutation (Tartaglia 2002; Jongmans 2005).

Zenker et al confirmed that *SOS1,* the gene encoding a guanine nucleotide exchange factor for Ras, is the second major gene for NS. Patients who carry mutations in this gene have characteristic features with frequent ectodermal anomalies such as keratosis pilaris and curly hair but generally normal development and linear growth (Tartaglia *et al.* 2007; Zenker 2007).

1.8.3 Genotype – phenotype correlation

A study by Tartaglia et al 2001, has suggested that individuals with Noonan syndrome due to mutations in *PTPN11* gene have a higher incidence of pulmonary valve stenosis (Tartaglia *et al.* 2001). However hypertrophic cardiomyopathy is less prevalent in those with mutations in this gene. Additionally studies have shown links between *PTPN11* gene mutations and phenotypic features such as short stature, pectus deformity, easy bruising and characteristic facial appearance (Zenker *et al.* 2004).

It was postulated that due to the presence of post- receptor signaling defects resistance to growth hormone therapy caused lack of response to short-term growth hormone (GH) treatment in *PTPN11* mutation-positive individuals. However it has now been proven that individuals with a *PTPN11* mutation present with more severe short stature and, therefore, reach a lower final height despite a similar height gain due to therapy (Noordam *et al.* 2008).

RAF1 gene had a striking correlation with hypertrophic cardiomyopathy, with 95% of individuals with mutations in this gene showing this feature while only 18% of those with NS without the mutation having this feature. One third of individuals with NS also presented with multiple nevi, lentigines, and/or café au lait spots (Pandit *et al.* 2007). Those with a mutation in the *KRAS* gene showed a greater degree of intellectual deterioration than others with NS without this mutation.

1.9 Justification

TS is a social burden to a family because of the phenotype of those patients and their inability to attain menarche and have children. Even though there are many other problems associated with TS, those are the main worries of the patients and their families in Sri Lanka. It will be a benefit for the patient if we could give the correlation between the cytogenetic abnormality and the clinical features, to the patients with the genetic diagnosis. That will help them to diagnose other medical and surgical problems associated with TS early and take appropriate action. So far characterization of cytogenetic abnormalities in patients with TS has not been done in Sri Lanka. An important aspect of this study is the identification of the genetic abnormalities and the genotype-phenotype correlations associated with TS in Sri Lankan patients. This will facilitate early genetic diagnosis, provide accurate genetic counselling and guide appropriate treatment. Molecular genetic testing to identify NS which is the main differential diagnosis of TS is still not available in Sri Lanka. As a start, 4 out of 15 exons of the *PTPN11* gene which is a common gene causing NS will be screened in this study. The selected exons have the highest frequency of reported mutations (hot spots). The *PTPN11* gene defects that would be identified would enable genetic diagnosis to be made in patients whose karyotypes do not indicate TS. This would also enable proper genetic counselling and long term management of such patients.

1.10 Objectives

Main objective of this study is to identify the cytogenetic variations of TS and correlate them with the phenotypic pattern of the patients.

- To determine the genetic etiology in patients with TS
- To examine phenotypic pattern in patients with TS
- To correlate the genetic etiology with the clinical phenotype in patients with TS

- To determine *PTPN11* gene mutation in patients whose karyotype does not indicate TS

2. METHODOLOGY

All the methods used for the investigation will be discussed in this chapter. That includes ethical considerations, recruitment of the patients, the clinical examination, methods of analysis of the *PTPN11* gene mutation and statistical analysis of the results.

2.1 Ethical considerations

This study was conducted according to the Declaration of Helsinki (2008) and received ethical clearance from the Ethics Review Committee (ERC) of the Faculty of Medicine, University of Colombo, Sri Lanka. This study has social value because it helps to the advancement of the knowledge on the cytogenetic variation in TS and their phenotypic correlation that will be useful in the future practice of clinical genetics and genetic counselling in Sri Lanka. The study was designed appropriately to ensure scientific validity. The study was open to all patients with a clinical diagnosis of TS and thus, there was fair participant selection. Appropriate measures were taken to ensure that consent was obtained in an ethical manner from all study participants. Written informed consent was obtained from all study participants using consent forms in Sinhala and Tamil languages. In the case of children, written informed consent was obtained from the parents or in the absence of the parents, proxy consent was obtained from the child's guardian. Each participant was given an information sheet which included details about the study and a consent form to be read and signed before participating in the study. All study participants or in the case of children, their parents/guardians were able to read and understand the information and make a voluntary decision to participate in the study. If the participants/parents/guardians had any questions about the study, they were answered before signing the consent form. Every participant was informed that they are free to withdraw his/her consent to participate in the study at any time, with no penalty or effect on medical care or loss of benefits. All participants were given contact details of the investigators and the ERC for clarification of any doubts about the

study. To ensure privacy, the participants/parents/guardians were interviewed privately in the genetic counselling room at HGU and they were able to discuss privately with the investigators without the presence of others. Written informed consent was obtained after providing the necessary information and giving them time to make a decision in private. All adult study participants were examined in the privacy of the examination room at HGU. The data collection booklet was designed to protect confidentiality of information gathered. Soon after collecting the personal information, the identification page was removed and filed separately. The only identification number in the rest of the booklet was a coded subject study number. That could not be linked to an individual without the page containing the personal information and that was kept separately locked by the principal investigator. The electronic database containing the data only had the subject study number which ensures confidentiality. The database and the computer containing the database were password protected. These measures ensured that confidentiality was highly protected. Any information was not released or published by which the participants can be identified. The data were not used in a way that the participants would be identified in any public presentation or publication.

The minimal risk of pain or minor bruising at the venepuncture site was minimized by performing the venepuncture by a trained phlebotomist under aseptic conditions. There was a direct benefit to some study participants by participating in this study. They would become aware whether they have a genetic mutation in the *PTPN11* gene and associated genetic risk. As genetic testing for this disorder is not freely available in Sri Lanka, this knowledge would be valuable to the patients. The patients who had a genetic diagnosis of TS were examined thoroughly to get their phenotypic pattern and they were benefitted by that many of them had undiagnosed defects as they were not followed up properly. In addition, study participants and parents/guardians would receive genetic counselling based on the results of the genetic

tests and would be referred for further long term management accordingly. This study would contribute to increasing knowledge on cytogenetic abnormalities and the phenotypic correlation in Sri Lankan patients with TS and the mutations in *PTPN11* gene. In addition, there was also the benefit of identifying a genotype-phenotype correlation in patients with NS that will be useful in the future practice of clinical genetics and genetic counseling. When the common genetic defects causing NS in Sri Lankan patients are known, it will be possible in future to develop cheap genetic tests suitable for use in Sri Lanka to diagnose this disorder. Once results of this study are published, a written summary of the results, in lay language would be posted to all participants. The samples and data would be stored for future use after the completion of the study for further studies in NS. Appropriate consent would be obtained for this purpose and such studies would be subject to ethics review prior to commencement.

2.2 Recruitment of subjects

This was a descriptive study and 57 patients with a clinical diagnosis of TS were recruited retrospectively. They were identified from the clinical records of the patient database maintained at the HGU from January 2006 onwards. They were contacted via phone/mail and the information sheets were given to them. Those who gave informed consent were recruited into the study.

2.2.1 Study participants

2.2.1.1. Inclusion Criteria

Patients who met all of the following criteria were recruited into the study.

- Phenotypic Sex – Females with a clinical diagnosis of TS
- Ability to provide written informed consent or in the case of children, the availability of a parent/guardian who can provide written informed consent.

2.2.1.2. Exclusion Criteria

Participants who met any of the following criteria were excluded from the study.

- Patients who are unable to provide written informed consent.
- Children with parents or guardians who are unable to provide written informed consent.

2.3 Clinical Evaluation

At recruitment study participants or in the case of children, parents/guardians were personally interviewed by the principal investigator to obtain demographic and clinical data. Complete medical, surgical and gynaecological history was obtained from each participant. Additional clinical data was also gathered by examining the participants' medical records. The family pedigree included familial background up to 3 generations whenever possible. Detailed family history was documented regarding family history of short stature, primary amenorrhoea, secondary amenorrhoea and subfertility in other siblings, family members or relatives. Complete physical examination of all the patients was done. Height/ length of all the patients were measured and stature was categorized into normal or short stature using standard height for age charts. Height less than the 10^{th} centile was taken as short stature. All the patients were tested for strabismus by cover uncover test and for colour vision by Ishihara charts. Visual acuity was checked by the use of Snellen's chart. Gross hearing assessment was performed on each patient to test the hearing status. Development of breasts and secondary sexual features such as pubic hair were classified according to Tanner stages. In view of testing cognitive function and visual spatial skills, Mini Mental State Examination (MMSE) and executive function screen including Clock Drawing Test was performed.

2.4 Biological samples and genetic testing

2ml of blood was taken from the patients whose karyotypes did not indicate TS. Blood was not taken from the patients who have a TS positive karyotype. These blood samples were stored at -80°C. DNA was then extracted from the samples and molecular genetic tests were performed. These tests included Polymerase Chain Reaction and DNA sequencing of the *PTPN11* gene according to published methods (Kosaki *et al.* 2002).

2.4.1 DNA extraction

Blood from the 24 patients whose karyotypes were negative for Turner syndrome was collected into EDTA containing tubes and stored at -80°C prior to DNA extraction. DNA extraction was done using Promega Wizard® Genomic DNA purification kit according to the manufacturer's protocol as follows: 300 µl of whole blood was added to 900 µl of cell lysis solution. The mixture was incubated for 10 minutes at room temperature to lyse the red blood cells. Following incubation, the mixture was centrifuged briefly at 14,000 rpm for 20 seconds. Then the supernatant was discarded without disturbing the white pellet formed. This was followed by vigorous vortexing for about 10-15 seconds until the white blood cells were resuspended. Next 300 µl of nuclei lysis solution was added to the microcentrifuge tubes containing the resuspended cells. This solution was pipetted 5-6 times to lyse the white blood cells. The extraction process was continued by adding 1.5 µl of RNase solution to the nuclear lysate, incubating at 37°C in a water bath for 15 minutes and by cooling back to room temperature. This was then followed by adding 100 µl of protein precipitation solution and vortexting vigorously for 10-20 seconds. This was followed by centrifugation at 14,000 rpm for 3 minutes and the supernatant was transferred to 1.5µl microcentrifuge tubes containing 300 µl of room temperature isopropanol. This mixture was then gently mixed until thread-like strands of DNA form a visible mass. This was again centrifuged at 14,000 rpm for 1 minute and the supernatant was discarded. Next 300 µl of room temperature 70% ethanol was added

to the DNA and the tubes were gently inverted to wash the DNA pellet and the sides of the microcentrifuge tubes. Then the ethanol was carefully aspirated and the tubes were inverted on clean absorbent paper and the pellets were air-dried overnight. Lastly, 100 µl of DNA rehydration solution was added to the microcentrifuge tubes containing DNA and the DNA was rehydrated by incubating the solution one hour at 65°C. The eluted DNA samples were labeled and stored at -20°C. These samples were used for PCR experiments and that will be described next.

2.4.2 Polymerase chain reaction

Amplification of selected DNA segments of interest was done using the polymerase chain reaction (PCR). PCR amplification involves simultaneous primer extension on complementary strands of DNA with two oligonucleotide primers which are specific to each strand flanking the genomic region to be amplified. This was carried out using thermostable *Taq* DNA polymerase enzyme in the presence of deoxynucleotides and a reaction buffer containing Mg^{2+}. All PCR primers and buffers used in these investigations were from Integrated DNA Technologies (IDT) USA.

Out of 15 exons of the *PTPN11* gene primers were designed to amplify four exons (3, 8, 9, and 13).

Primer pairs to amplify exons of PTPN11 were shown in this table below. (Table 2.1) (Tartaglia 2002)

Primer pairs and annealing temperatures used to amplify exons of *PTPN11* gene and sizes of PCR products are shown in this table below (Table 2.1). Adapted from Tartaglia *et al.* (2002) "*PTPN11* Mutations in NS: Molecular spectrum, Genotype – Phenotype correlation, and Phenotypic Heterogeneity." Am. J. Hum. Genet. 70:1555-1563 2002

Exon	Forward primer	Reverse primer	Size (bp)	Annealing temperature (C)
3	NS5 CTTGCCTCCCTTTCCAATGGAC	NS6 GCATTTCTGACACTCAGGGCAC	290	58
8	NS15 GACTAGGCTGGGGAGTAACTG	NS16 GCTAGAAATTTAGGAAGAAAATCCTTCAAACACC	294	58
9	NS17 CAGTGTTTTCTGACCATACATTTCTAGCC	NS18 CATGGCCAATCTGACATGTCTGATAC	337	58
13	NS25 GTCTCTGAGTCCACTAAAAGTTGTGC	NS26 AGCGTATCCAAGAGGCCTAGC	303	58

First, 100ng of genomic DNA was amplified using PCR in a 25 µl volume containing, 200 µM of each deoxynucleotides (dNTPs: dATP, dCTP, dGTP and dTTP), 0.5 µM of each forward and reverse primer, 2.5 mM $MgCl_2$, 1U Go*Taq* DNA polymerase enzyme (Promega), PCR Buffer. PCR amplification included repeated cycles of heat which were performed in a thermal cycler (ABI 2720) with the following conditions: following initial denaturation at 94°C for 5 min, amplification was performed using 35 cycles at 94°C for 40 s, annealing for 40 s under the optimised temperature, and extension at 72°C for 60 s and final extension 72°C for 10 minutes. No template control was included in the PCR analysis to rule out carryover contamination. At the end of the process the presence of amplified PCR products were confirmed by subjecting agarose gel electrophoresis.

2.4.3 Agarose gel electrophoresis

Electrophoresis through agarose is the standard method used to separate and identify DNA fragments. This was performed by moving negatively charged DNA molecules towards the anode through an agarose gel under the electric field. The location of the amplified PCR product within the agarose gel was determined directly by staining with low concentrations of ethidium bromide dye which intercalates with DNA as it fluoresces when examined under

UV transilluminator. Agarose gels were prepared by mixing an appropriate volume of 1x TBE buffer and an appropriate amount of molecular biology grade agarose. This helps to obtain the correct percentage gel (weight/volume). 0.5 µg/ml of ethidium bromide was also added to each gel. Gels were poured into gel casting trays fixed with combs with the desired number of wells and allowed to set at room temperature. When the gels were set it was submerged in 1x TBE buffer and the PCR products which were mixed with gel loading dye were loaded into the wells of the agarose gel along with the 100bp marker. The gel electrophoresis was carried out using an appropriate level of current. Subsequently the PCR products were visualized by observing the agarose gel under the UV transilluminator. This should give a single band if the PCR has worked as expected and has amplified a single segment of the target DNA.

2.4.4 Automated sequencing

The PCR products were purified using ExonucleaseI (ExoI) and Shrimp Alkaline Phosphatase (SAP) (Fermentas) according to the manufactures instructions. The DNA sequencing was performed with the PCR primers using the ABI PRISM BigDye® Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA). Sequencing fragments were purified using Centriflex® gel filtration cartridge Edge Biosystem (USA). Sequences of the purified products were analyzed using an ABI 3130 Genetic Analyzer. This system works on the basis of the Sanger dideoxy chain termination principle. It contains four ddNTPs with different fluorescence labels and AmpliTaq® DNA polymerase. At the end of the reaction PCR products of varying sizes all terminating with a fluorescent-labelled dideoxy nucleotide are generated. The sequence is then read out automatically by capillary electrophoresis of cycle sequencing products through an automated sequencing machine. DNA sequencing analysis was done using Chromas Lite 2.01 software.

2.5 Software tools and electronic database information

At recruitment all the patients were interviewed and examined. Their clinical features, birth history, past medical and surgical history, family history and all the investigation reports were entered into an electronic data base. Personal data of the patients were kept separately from the data base and it was maintained only by the investigators to secure confidentiality. Gen Bank at the National Centre for Biotechnology Information (NCBI), USA was searched to obtain DNA sequences and gene mapping information which is a free database that can be accessed online by URL: http://www.ncbi.nlm.nih.gov. The support from the already existing data bases related to *PTPN11* mutation were taken to detect mutations. The Human Genome Mutation Database (http://www.hgmd.cf.ac.uk/ac/gene.php?gene=PTPN11), the Human Genome Variation Database (http://www.hgvs.org/dblist/dblist.html), Uniprot (http://www.uniprot.org/) and *PTPN11* base mutation browser (http://bioinf.uta.fi/PTPN11base/index.php?content=pubs/SH2) were referred to detect previously reported mutations.

UCSC Genome Browser BLAT tool (http://genome.ucsc.edu/cgi-bin/hgBlat?command=start) was used to align the reference sequences of unreported mutations. A tool which predicts possible impact of an amino acid substitution on the structure and function of a human protein named PolyPhen-2 (Polymorphism Phenotyping version 2) (http://genetics.bwh.harvard.edu/pph2/) was used to identify the significance of the mutations found by sequencing.

2.6 Statistical methods

Phenotypic data which were entered directly into a database were manually verified for errors. In the phenotypic analysis, summary values were presented as mean [standard deviation (SD)] when data were normally distributed and as median when not. The statistical

significance of the frequency was analyzed by Fisher's exact probability test and $P < 0.05$ was considered significant.

3. RESULTS

3.1 Demographic characteristics

Thirty three patients with a genetic diagnosis of TS were examined. Their ages varied from 1 year to 28 years. Ages were calculated from their date of birth to the date of presentation for karyotyping at the Human Genetics Unit. Mean age at presentation was 14.6 (SD± 7.6) years and the median age was 15 years. Age group distribution of TS patients is shown in Figure 3.1. Highest percentage was in the 11 - 15 age group (33.3%) followed by the 16 - 20 age group (24.2%)

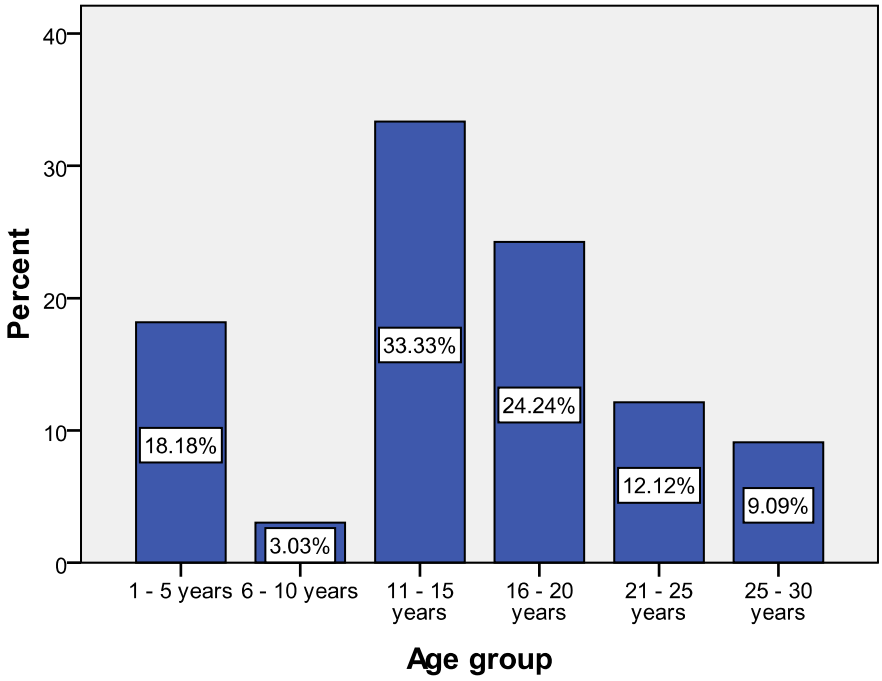

Figure 3.1: Age group distribution of Turner syndrome patients

Out of the 33 patients, 27 patients (81.8%) had the indication for karyotyping as suspected Turner syndrome. Turner mosaicism was the indication for karyotyping in 2 patients (6.1%). Three patients (9.1%) had the indication as primary amenorrhoea. Only 1 patient (3%) had the indication as secondary amenorrhoea.

3.2 Genetic etiology of TS

The cytogenetic abnormalities of the study population are shown in Figure 3.2. Out of the 33 patients, Monosomy X (45,X) karyotype constituted the highest percentage (39.4%) followed by 46, XX/45,X (Turner mosaic) (33.3%).

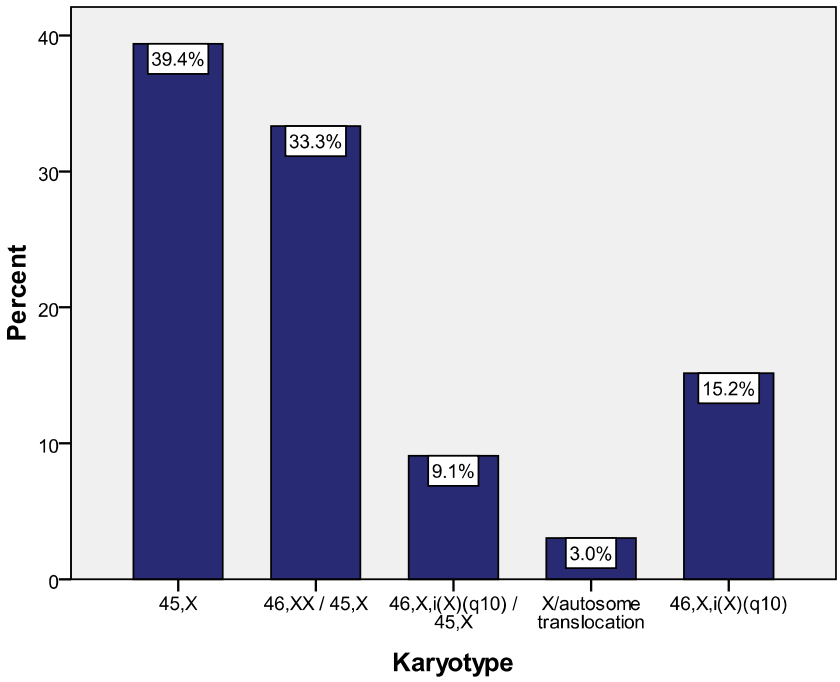

Figure 3.2: Percentage distribution of the cytogenetic abnormalities in patients with Turner syndrome

The karyograms identified in this study are shown in the following figures.

A karyogram of a patient with classical TS (Monosomy X/ 45,X) is shown in Figure 3.3.

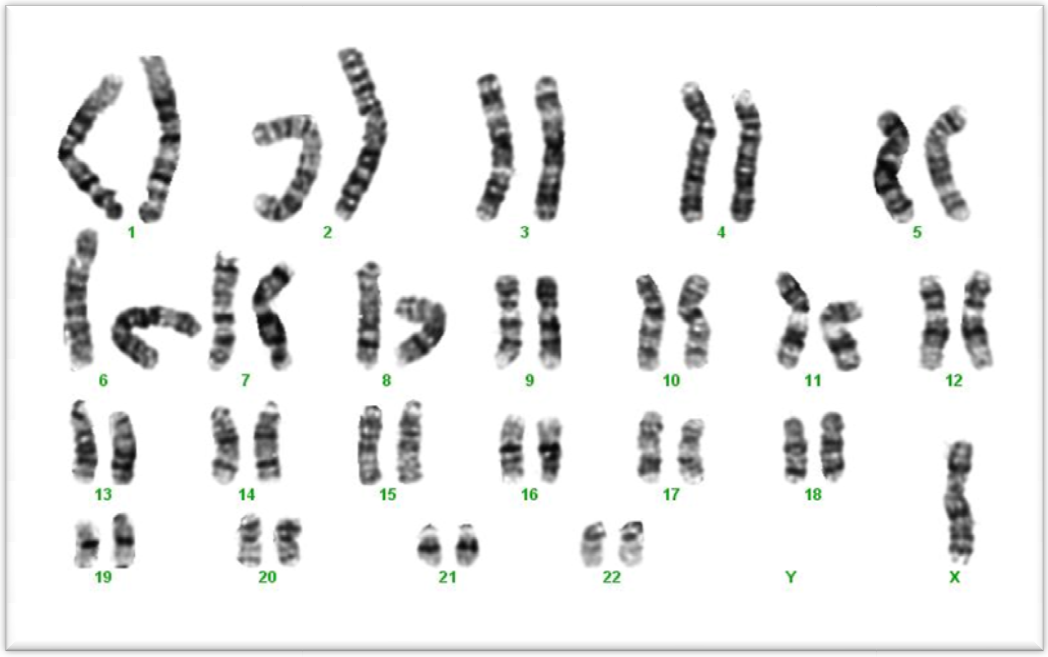

Figure 3.3: Karyogram of a patient with classical TS

Figure 3.4 shows a karyogram of a patient with Turner mosaicism (46, XX/45, X).

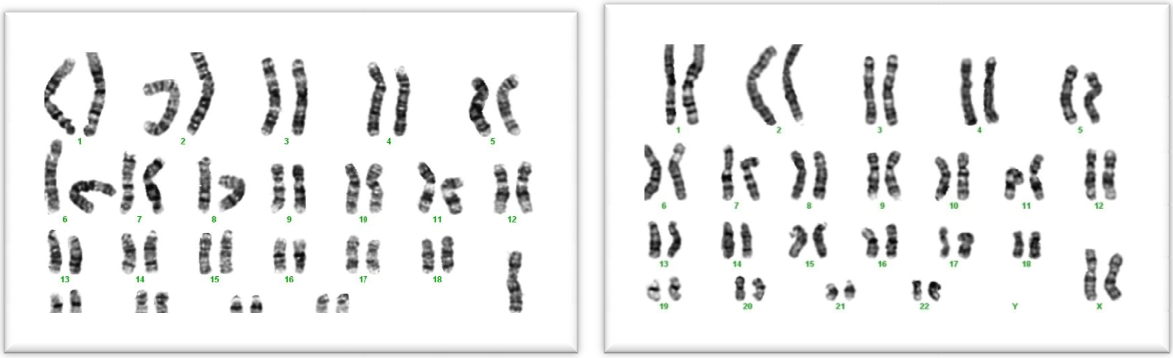

Figure 3.4: Karyogram of a patient with mosaic TS

Figure 3.5 shows a karyogram of a patient with Isochromosome TS [46, X, i(X) (q10)].

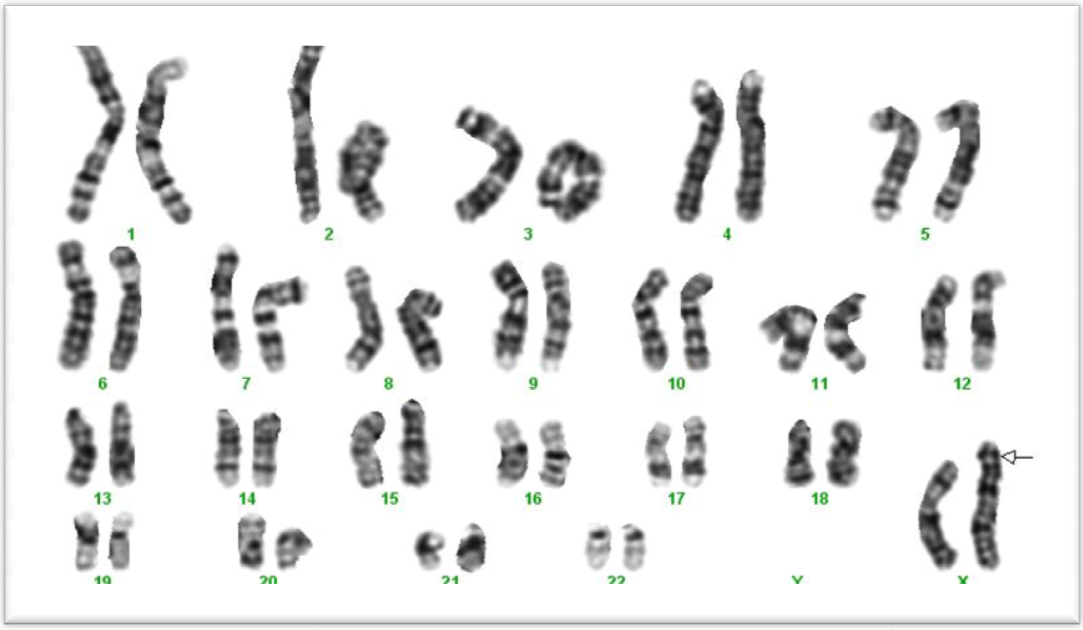

Figure 3.5: A karyogram of a patient with Isochromosome TS

Figure 3.6 shows a karyogram of a patient with Turner mosaicism for X isochromosome and Monosomy X.

Figure 3.6: A karyogram of a patient with Turner mosaicism for X isochromosome and Monosomy X

Figure 3.7 shows the karyogram of the patient with a translocation between chromosome X and an autosome. Chromosome 13 is suspected to be the autosome involved in the

translocation. This is to be further characterized using advanced molecular cytogenetic techniques.

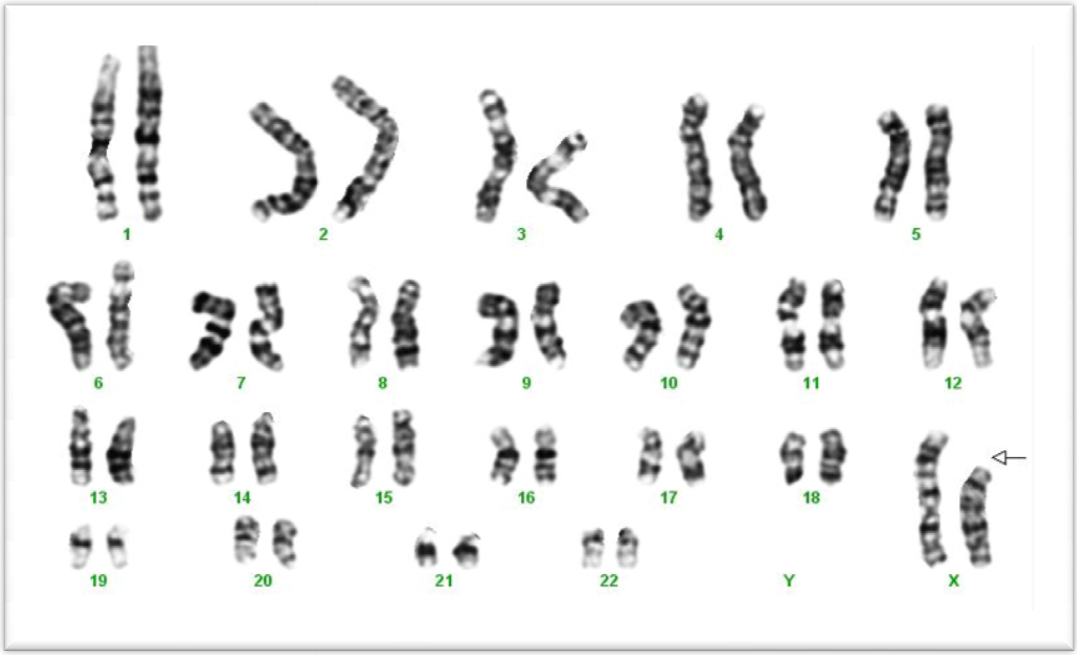

Figure 3.7: The karyogram of the patient who had a translocation between chromosome X and an autosome

3.3 Phenotypic pattern of TS

No patient had a family history of short stature, primary amenorrhea or subfertility. Only 2 (6.1%) patients had consanguineous parents.

Ante natal ultra sound scan was done in only 8 patients (24.2%). Out of those 8 patients, horse shoe kidney was detected in one patient and other features like cardiac defects and cystic hygroma were not detected.

Phenotypic features were categorized according to the age periods they manifested i.e. post natal, childhood and adolescent period. These are summarized in tables as follows. Table 3.1 shows the percentage distribution of the features seen in the post natal period. Extra skin around the neck was the most common feature seen in this period.

Table 3.1: Percentage distribution of post natal phenotypic features

Phenotypic feature	frequency	percentage
Dorsal oedema of the hands and feet	3	9.1%
Extra skin around the neck	4	12.1%
Cystic hygroma	1	3%

Table 3.2 summarizes the percentage distribution of features manifesting during childhood. Short stature (81.8%), webbed neck (57.5%), and high arched palate (90.9%) were the most common features seen in childhood.

Table 3.2 Percentage distribution of childhood phenotypic features

Phenotypic feature	frequency	percentage
Short stature	27	81.8%
Webbed neck	19	57.5%
Low posterior hairline	13	39.4%
High arched palate	30	90.9%
Low set ears	7	21.2%
Prominent ears	6	18.2%
Strabismus	5	15.2%
Ptosis	4	12.1%
Ptosis and strabismus	2	6.1%
Epicanthal folds	2	6.1%
Short fingers and toes	8	24.2%
Short 4^{th} metatarsal bone	9	27.3%
Short 4^{th} metacarpal bone	2	6.1%
Wide carrying angle	27	81.8%
Hyper convex nails	7	21.2%

In Table 3.3 the features manifesting during adolescence are summarized. There is an overlapping between the features manifesting during childhood and adolescence. The commonest manifestation during adolescence was primary amennorhoea found in 75.8%.

Table 3.3: Percentage distribution of features seen in adolescence

Phenotypic feature	Frequency	Percentage
Primary amenorrhoea	25	75.8%
Shield shaped chest	23	69.7%
Widely spaced nipples	24	72.7%

Other than the phenotypic manifestations, medical problems which are commonly found in TS were also recorded. They are listed in Table 3.4.

Table 3.4: Percentage distribution of medical problems seen in TS patients

Medical problem	Frequency	Percentage
Middle ear infections	11	33.3%
Frequent urinary tract infections	4	12.1%
Vision impairment	7	21.2%
Bone fractures	2	6.1%

Hearing problems and multiple pigmented naevi were not seen in any of the patients. Among the study population only 7 (21.2%) patients had hyper convex nails and other 26 (78.8%) patients had normal nails. Although endocrine disorders such as diabetes mellitus (DM) and hypothyroidism are common in TS, none of the patients in this study had diabetes mellitus. Only five patients (15.2%) had hypothyroidism and were on thyroxine. Cognitive functions were measured by assessing visual spatial skills using the clock drawing test. Out of the 33 patients, 5 were not able to perform the test. Out of 28 who performed the test, 14 (50%)

patients were normal and the other half were cognitively impaired. There was bilateral impairment of visual acuity in 10 (30.3%) patients and unilateral in 3 (9.1%) patients.

Ultrasonically normal uterus was seen in only 8 (24.2%) patients and normal ovaries were seen in only 6 (18.2%) patients. None of the patients had undergone testing for antithyroid antibodies. Echocardiography was performed in 19 patients. Cardiac abnormalities were detected in 5 (15.2%) patients while in 14 (42.4%) it was normal. The detected abnormalities were aortic stenosis with mild aortic regurgitation, mitral valve prolapse, ventricular septal defect, atrial septal defect and bicuspid aortic valve.

3.4. Variation between the genetic etiology and the clinical phenotype

Frequencies of phenotypic features according to the cytogenetic abnormality are summarized in following tables.

Table 3.5 shows the percentage distribution of cytogenetic abnormality of post natal features.

Table 3.5: Percentage distribution of cytogenetic abnormality associated with post natal features

Karyotype	Dorsal oedema of the hands and feet	Extra skin around the neck	Cystic hygroma
45,X	3 (100%)	3 (75%0)	1 (100%)
46,XX/45,X	0	0	0
46,X,i(X)(q10)	0	0	0
46,X,i(X)(q10)/45,X	0	0	0
X/autosome translocation	0	1(25%)	0
Total	100%	100%	100%

Among 19 patients who had a webbed neck, 10 (52.6%) were from the classical Turner group followed by Turner mosaic and patients with isochromosome Xq TS. Table 3.6 shows the percentage distribution of webbed neck according to the karyotype.

Table 3.6: Percentage distribution of patients with webbed neck according to the cytogenetic abnormality

Karyotype	Webbed neck
45,X	10(52.6%)
46,XX/45,X	8(42.1%)
46,X,i(X)(q10)	1(5.3%)
46,X,i(X)(q10)/45,X	0
X/autosome translocation	0
Total	19(100%)

Table 3.7 shows the height for age distribution in patients with TS according to the cytogenetic abnormality.

Table 3.7: Percentage distribution of patients with short stature and normal stature according to the cytogenetic abnormality

Karyotype	Short stature	Normal
45,X	12(44.4%)	1(16.7%)
46,XX/45,X	6(22.2%)	5(83.3%)
46,X,i(X)(q10)	5(18.5%)	0
46,X,i(X)(q10)/45,X	3(11.1%)	0
X/autosome translocation	1(3.7%)	0
Total	100%	100%

Table 3.8 shows the distribution of facial features according to the karyotype.

Table 3.8: Percentage distribution of patients with facial features according to the cytogenetic abnormality

Karyotype	Low posterior hairline	High arched palate	Low set ears	Prominent ears
45,X	9(69.2%)	13(43.3%)	1(14.3%)	4(66.7%)
46,XX/45,X	2(15.4%)	10(33.3%)	5(71.4%)	1(16.7%)
46,X,i(X)(q10)	1(7.7%)	4(13.3%)	1(14.3%)	0
46,X,i(X)(q10)/45,X	0	2(6.7%)	0	1(16.7%)
X/autosome translocation	1(7.7%)	1(3.3%)	0	0
Total	100%	100%	100%	100%

Table 3.9 shows the distribution of ophthalmic features according to the karyotype

Table 3.9: Percentage distribution of patients with ophthalmic features according to the cytogenetic abnormality

Karyotype	Strabismus	Ptosis	Ptosis and strabismus	Epicanthal folds
45,X	2(40%)	4(100%)	1(50%)	2(100%)
46,XX/45,X	2(40%)	0	0	0
46,X,i(X)(q10)	0	0	1(50%)	0
46,X,i(X)(q10)/45,X	0	0	0	0
X/autosome translocation	1(20%)	0	0	0
Total	100%	100%	100%	100%

Table 3.10 summarizes the percentage distribution of orthopedic features according to the karyotype in patients with TS. Patients with classical TS had the highest percentage of orthopedic features followed by Turner mosaics.

Table 3.10: Percentage distribution of orthopedic features according to the cytogenetic abnormality in patients with TS

Karyotype	Short fingers and toes	Short 4th metatarsal bone	Short 4th metacarpal bone	Wide carrying angle	Madelung deformity
45,X	3(42.9%)	4(44.4%)	1(50%)	12(44.4%)	0
46,XX/45,X	2(28.6%)	3(33.3%)	1(50%)	7(25.9%)	0
46,X,i(X)(q10)	1(14.3%)	0	0	4(14.8%)	0
46,X,i(X)(q10)/45,X	1(14.3%)	2(22.2%)	0	3(11.1%)	0
X/autosome translocation	0	0	0	1(3.7%)	1(100%)
Total	7(100%)	100%	100%	100%	100%

Table 3.11 shows the distribution of adolescent features according to the karyotype.

Table 3.11: Percentage distribution of adolescent features in patients with TS according to the cytogenetic abnormality

Karyotype	Primary amenorrhoea	Shield shaped chest	Widely spaced nipples
45,X	13(52%)	12(52.2%)	13(54.2%)
46,XX/45,X	7(28%)	7(30.4%)	6(25%)
46,X,i(X)(q10)	3(12%)	3(13%)	3(12.5%)
46,X,i(X)(q10)/45,X	2(8%)	1(4.3%)	2(8.3%)
X/autosome translocation	0	0	0
Total	100%	100%	100%

Out of 8 patients who had attained menarche, there were no patients with classical Turner and all were Turner mosaics and the other variants. Among the seven patients who had hyper convex nails, 3 (42.9%) were from Turner monosomy group followed by 3 (42.9%) from the isochromosome mosaic and 1 (14.3%) patient from isochromosome group. Clock drawing test was done to get an idea about cognitive functions of the patients. It could not be done by 5 patients due to their age. From the 14 cognitively deficient patients 6 (42.9%) were from the 45, X karyotype, followed by 4 (28.6%) Turner mosaics, 2 (14.3%) patients with isochromosome, 1 (7.1%) patient with isochromosome mosaic and 1 (7.1%) patient with X/autosome translocation.

Table 3.12 shows the distribution of cognitive functions according to the karyotype.

Table 3.12: Percentage distribution of cognitive functions according to the cytogenetic abnormality in patients with TS

Karyotype	Cognitively deficit	Cognitively normal
45,X	6(42.9%)	4(28.6%)
46,XX/45,X	4(28.6%)	5(35.7%)
46,X,i(X)(q10)	2(14.3%)	3(21.4%)
46,X,i(X)(q10)/45,X	1(7.1%)	2(14.3%)
X/autosome translocation	1(7.1%)	0
Total	100%	100%

Out of 5 patients who had cardiac abnormalities 3 (60%) were from the classical Turner group and 2 (40%) were from the Turner mosaic group. Regarding the ultra sound scan (USS) findings, in patients with classical TS out of 12 those who have undergone USS, only 1 patient had a normal uterus. In patients with Turner mosaic karyotype out of 11 patients 6 had normal uterus. In all 3 patients with the karyotype of 46, X, i(X) (q10) /45, X had small uterus. In patients with 46, X, i(X) (q10) karyotype not a single patient had a normal uterus. The patient who had X/ autosome translocation had a normal uterus in the USS. Considering the USS findings of ovaries of each patient group only one patient with classical TS had normal ovaries. But 4 patients with Turner mosaic karyotype got normal ovaries. No patients with Turner isochromosome or Turner isochromosome mosaic karyotypes had normal ovaries. In 11 patients out of all 33 ovaries were not visualised. There were no comments about the ovaries in 7 patients.

3.5. Correlation of the phenotype with the cytogenetic abnormalities in patients with TS

Patients were classified into 2 groups based on the karyotype to see whether there was significant variation in the phenotype between the Classical TS (Turner monosomy) and the other TS variants.

Results are summarized in the Table 3.13. Primary amenorrhoea, low posterior hair line, ptosis, shield shaped chest and widely spaced nipples were significantly associated with classical TS.

Table 3.13: Comparison of phenotypic features in patients with classical TS and TS variants

Phenotypic feature	Classical TS (n = 13)	TS variants (n = 20)	P value
Primary amenorrhoea	13(52%)	12(48%)	0.012
Hypothyroidism	2(40%)	3(60%)	1
Short stature	12(44.4%)	15(55.6%)	0.364
Webbed neck	10(52.6%)	9(47.4%)	0.087
Low posterior hair line	9(69.2%)	4(30.8%)	0.003
High arched palate	13(43.3%)	17(56.7%)	0.261
Shield shaped chest	12(52.2%)	11(47.8%)	0.05
Widely spaced nipples	13(54.2%)	11(45.8%)	0.005
Hyper convex nails	3(42.9%)	4(57.1%)	1.000
Increased carrying angle	12(44.4%)	15(55.65)	0.364
Ptosis	5(83.3%)	1(16.7%)	0.025
Epicanthal folds	2(100%)	0	0.148

3.6 *PTPN11* gene mutations in patients whose karyotypes did not indicate TS

After sequencing exon 3, 8, 9 and 13 of the *PTPN11* gene, 3 sequence variations were found.

1. The first was in exon 3 of patient number 45. This is reported to be a polymorphic variant located at position c.255 C>T (H85H) causing no change in the Histidine amino acid. The electropherogram of the sequence is shown in Figure 3.3 and the sequence variation is indicated by the arrow.

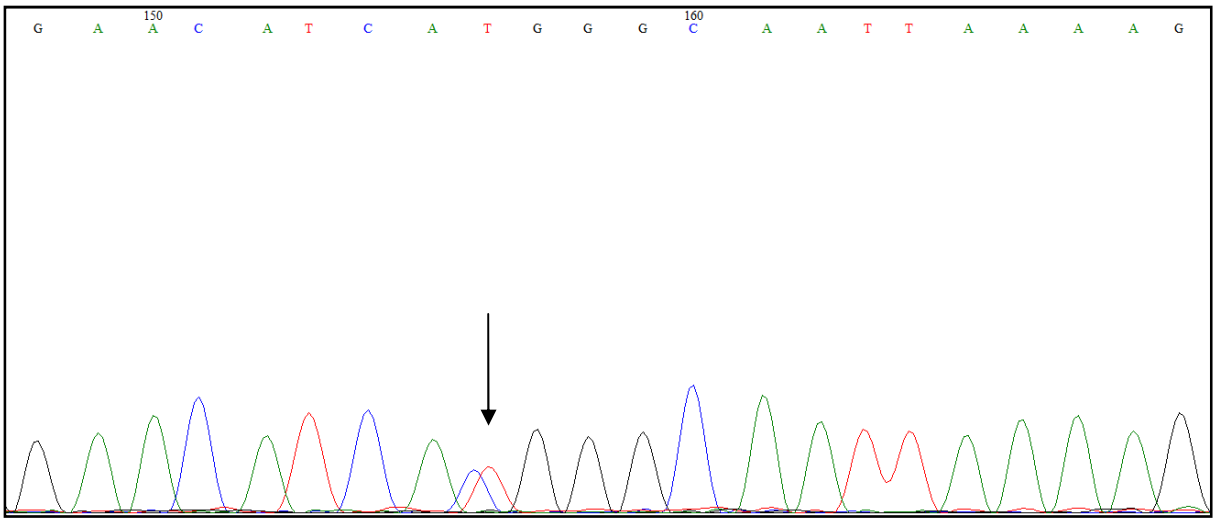

Figure 3.3: Electropherogram of the sequence showing c.255C>T in exon 3 of *PTPN11* gene

2. The second was in exon 9 patient number 2. It was located at the position c.1052 A>G (R351Q) and was not found in the scientific literature. The electropherogram of the sequence is shown in Figure 3.4 and mutation is marked by the arrow. It resulted in the alteration of an amino acid.

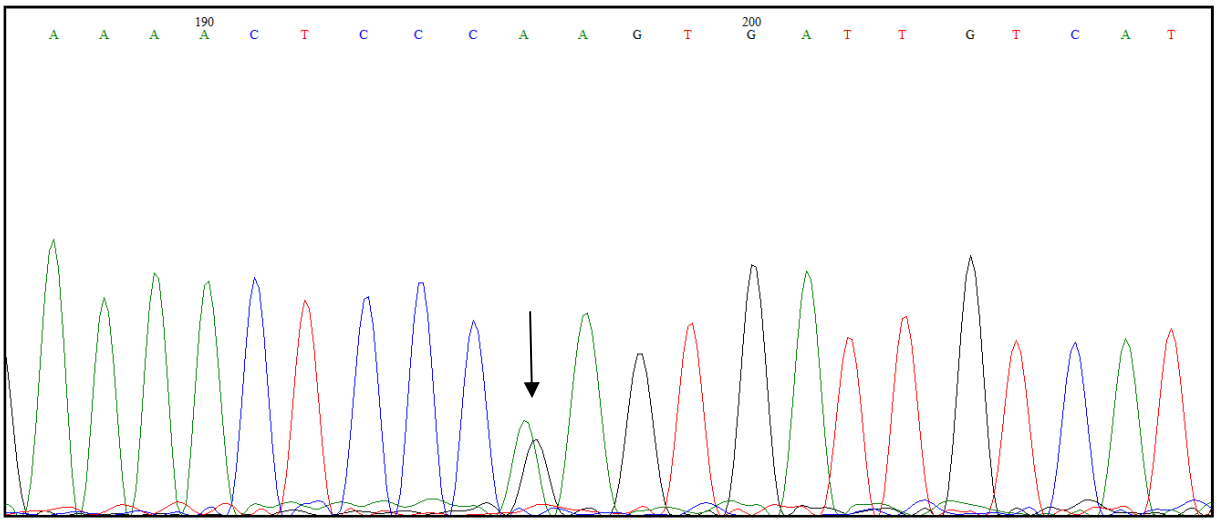

Figure 3.4: Electropherogram of the sequence showing c.1052A>G in exon 9 of *PTPN11* gene

The reference sequence was aligned with the tool BLAT in UCSC Genome Browser (hg19) and the amino acid was found to be conserved across species as shown in Figure 3.5.

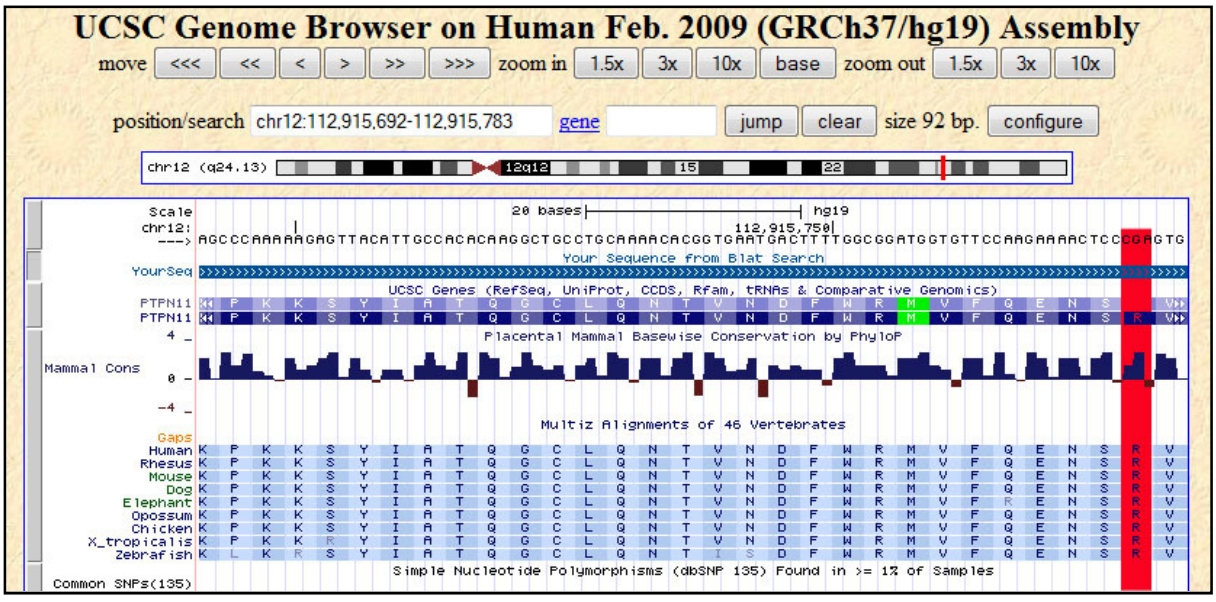

Figure 3.5: Image from UCSC Genome Browser showing the position of the mutation and the conservation of the amino acid (highlighted in red)

After analysing with PolyPhen version 2 bioinformatics tool, this mutation was predicted as benign (≤0.5 is Benign) with a score of 0.301 (sensitivity: 0.91; specificity: 0.89) and the output is shown in Figure 3.5.

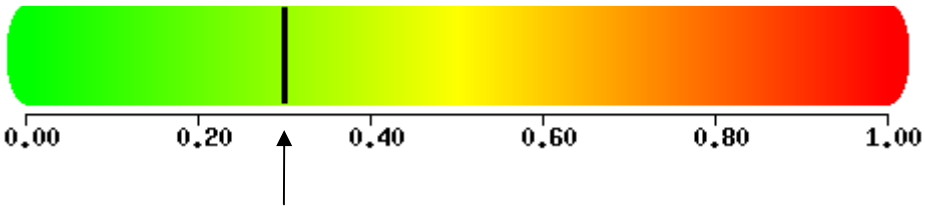

Figure 3.6: results of bioinformatics analysis using PolyPhen version 2

Location of the mutation and the 3D structure of the *PTPN11* protein are also given by this tool and it is shown in Figure 3.6. Location of the mutation is highlighted in the image on the right side.

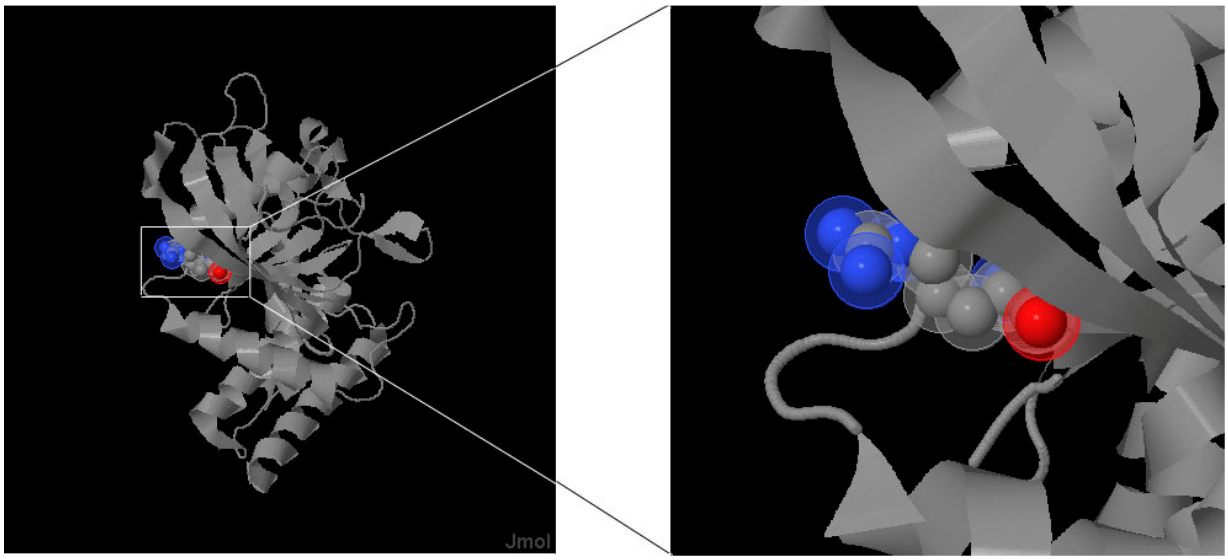

Figure 3.7: Location of the mutation and the 3D structure of the *PTPN11* protein

3. In patient number 37, a sequence variation was found in the intervening sequence between exon 8 and 9. It is located at the position c.934-27 T>C and was not found in the scientific literature. Electropherogram of the sequence is shown in Figure 3.8.

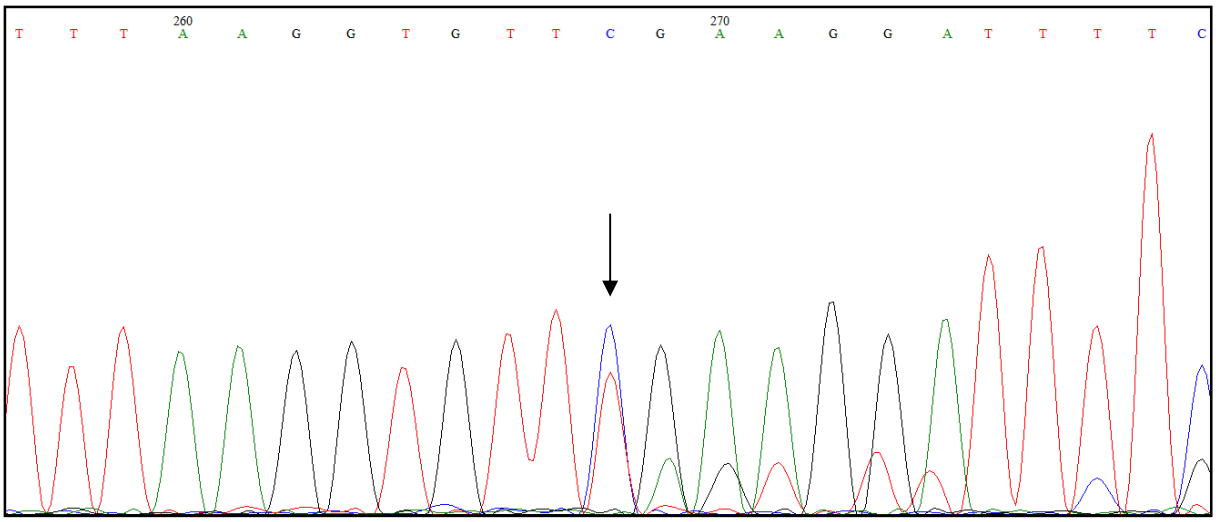

Figure 3.8: Electropherogram of the sequence showing c.934-27T>C in exon 9 of *PTPN11* gene

The reference sequence of this mutation was also aligned with the tool BLAT in UCSC Genome Browser (hg19) and the amino acid was found to be conserved across all species other than chicken and zebra fish as shown in Figure 3.9.

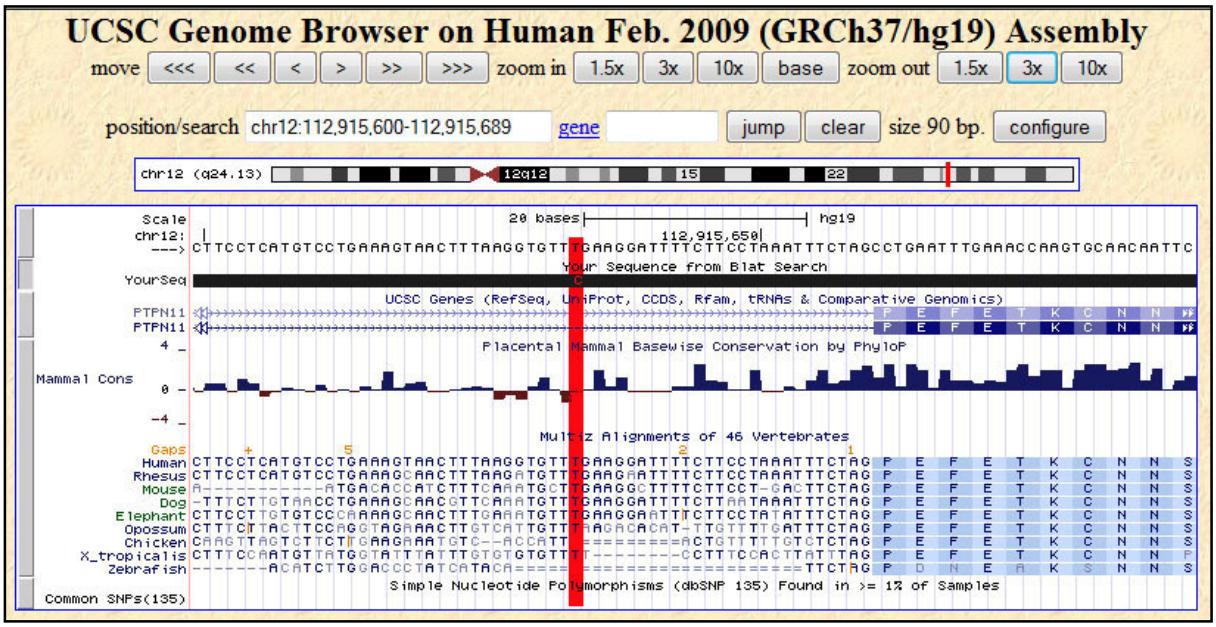

Figure 3.9: Image from UCSC Genome Browser showing the position of the mutation and the conservation of the amino acid

4. DISCUSSION

4.1 Demographic characteristics of the study population

The study population consisted of the patients who came to the Human Genetics Unit (HGU) with a clinical diagnosis of TS from 2006 onwards. Out of 57 patients who could be contacted and who were willing to participate for the research, 33 patients had a diagnosis of TS. Mean age of presenting was 14.6 years and the highest age group of presenting was 11 – 15 years followed by 16 – 20 years in this population. This is rather a late presentation as TS can be diagnosed at any time after birth. This could be due to the subtleness of the facial and other features in TS. Usually they tend to seek medical advice when there is a lack of pubertal changes and primary amenorrhoea. Though short stature is a very common feature in TS, in early ages it is difficult to identify as there are various other causes responsible for short stature other than TS (Morgan 2007).

According to Massa, G. et al in Belgium, more patients were diagnosed in infancy and early childhood and less in adolescence with a 22% of the girls were diagnosed after 12 years of age (Massa *et al.* 2005). Kerstine Stochholm et al also concluded that TS is diagnosed with a considerable delay of age in Denmark and the median age at diagnosis was 15.1 years similar to findings in this study (15 years) (Stochholm *et al.* 2006).

4.2 Genetic aetiology of TS

Cytogenetic variation was classified in the 33 patients who were recruited for the study. Among them highest percentage belonged to the classical TS group (39.4%) as expected. It was same in a descriptive study done at HGU in 1988 to see the chromosome anomalies in Sri Lanka, out of 76 patients 11 (14.5%) were TS patients and among them 9 (11.9%) were classical TS patients followed by 2 (2.6%) Turner mosaics (JAYASEKARA 1988). The reason for this could be the identification of more symptoms and signs in classical TS patients than in mosaic TS patients and other variants.

In a study done in Sweden it is shown that among the 112 TS females 41.1% were classical Turners, 23.2% were isochromosomes, 10.7% were Turner mosaics followed by 25% of other TS variants (Rizell *et al.*). But in Kammoun, I. et al. out of 89 TS patients participated in genetic analysis, Turner mosaicism was prevalent (47%) whereas classical Turners were 32% followed by other variants (21%) (Kammoun *et al.* 2008). In a study done in Siberia with 31 TS patients 15(48.4%) patients had classical TS and not like in Sri Lanka they were followed by isochromosome Turners with a frequency of 5 (16.1%) (Djordjevic *et al.*). But Suri, et al concluded that the most frequent karyotype was classical TS followed by mosaic TS and these findings in India are similar to our findings (Suri *et al.* 1995). In a study done in Northeastern Malaysia too classical TS accounted for 57.1% of patients with TS (Kannan *et al.* 2008).

4.3 Phenotypic pattern of TS

Phenotypic features of TS can be seen from the antenatal period onwards. Antenataly through an USS fetal oedema, cystic hygroma, loose nuchal skin, renal defects such as horse shoe kidney and cardiac defects could be identified in a fetus with TS (Sybert *et al.* 2004). Cystic hygroma is the well described and characteristic feature found in TS through USS (Loscalzo *et al.* 2005). In this study antenatal scan was performed in only 8 (24.2%) patients. Among these 8 patients only horseshoe kidney was detected in one patient. Not like in many areas of the world, in Sri Lanka ante natal screening for fetal abnormalities is not routinely available in the government sector. That may be the reason for lack of pre natal diagnosis of TS in the study population.

Though other phenotypic features of TS can be categorized according to the age of manifestation as post natal, childhood and adolescent, there is a considerable overlapping between these features.

In this study dorsal oedema of the hands and feet, presence of extra skin around the neck and cystic hygroma were the phenotypic features found in the post natal period. Out of 33 patients 4 (12.1%) had extra skin around the neck, 3 (9.1%) had dorsal oedema of the hands and feet and only 1 (3%) had cystic hygroma in the post natal period. Remaining 25 (75.8%) patients didn't show features during the post natal period. Though prematurity, birth weight and length also can be considered as characteristic features in newborn TS babies as reported by Hagman, *et al*.(Hagman *et al.* ; Bondy 2007), it is very difficult to do that in a retrospective analysis like this as most of the participants didn't have the records of length and the maturity.

The features found in childhood in Turner patients are short stature, short and webbed neck, low posterior hair line, low set ears, high arched palate, ophthalmic features such as ptosis, strabismus and other orthopedic features such as wide carrying angle, Madelung deformity, short 4^{th} metacarpals and metatarsals (Bondy 2007). Elsheikh, *et al*. reported that short stature is the commonest feature seen in the TS (Elsheikh *et al.* 2002). But in this study the commonest feature was high arched palate. Short stature and the wide carrying angle were the other common features. Webbed neck was also common in our population as in others. Though Madelung deformity is also frequently seen in TS patients, only one patient had that in this study group. Low posterior hair line was also seen in 39.4% of patients which is a very frequent feature in TS. Though it is said in the literature that multiple pigmented naevi are commonly found in patients with TS, in this study group they were not present in a single patient. That may be due to the dark complexion in Sri Lankans. According to a study done by Denniston *et al.* strabismus was a common ophthalmic feature (> 25%) in TS while ptosis and epicanthal folds were uncommon (5 – 25%) (Denniston *et al.* 2004). But in this study there were 5 (15.2%) patients with strabismus only, 4 (12.1%) patients with ptosis only and 2

(6.1%) with both features while only 2 (6.1%) had epicanthal folds, showing that those features are not very common in our country.

Features which can be seen during adolescence are the features that appear with puberty. As expected primary amenorrhoea was present in 25 (75.8%) patients and that was the commonest problem for seeking medical advice among patients in the late childhood and the adolescent period. Though the features like shield shaped chest and the widely spaced nipples can be seen in the childhood period, they are more prominently seen in the adolescent period and the adulthood. In the study population both the features were leading.

In patients with TS other than the mentioned phenotypic features there are various problems such as hearing, visual, endocrine, renal, autoimmune and cognitive problems. To test the hearing gross hearing test was done with the ambition of sending them for audiometry if some defect is detected with that. But nobody had a hearing impairment in the study population. There were few patients who had the audiogram report and they were also normal. In a study done in India with the participation of 45 TS patients, the results were the same as in this study and the reason they have given for that was not having the audiometry done in those patients (Suri *et al.* 1995). In this study also that may be the reason for the absence of hearing impairment. Though only 7 (21.2%) patients complained of vision impairment, after testing visual acuity, 13 (39.4%) of them were found to have impairment of visual acuity. Six patients among them didn't have any clue about the weakness in their eye sight.

Hypothyroidism and diabetes mellitus are the endocrine disorders commonly associated with TS (Gravholt 2004). In our study only 5 (15.2%) patients were found to have hypothyroidism and were on thyroxine. Screening tests were not performed in 14 patients. Even though thyroid antibody formation (auto immune thyroiditis) is very common among Isochromosome Turner patients, antithyroid antibodies were not tested in a single patient in

the study group (Gravholt 2004; Bondy 2007). Diabetes mellitus (DM) was also not found in this study population. The reason may be that patients who participated in this study were below 30 years of age and only 8 patients had been screened for DM out of 33. That suggests proper screening programmes for associated disorders should be developed in Sri Lanka. It is said that non insulin dependant diabetes mellitus is 2 – 4 times more common in TS than the normal females and also it may appear in an early age (Lichiardopol *et al.* 2007). Cardiac abnormalities found in TS are congenital heart defects such as bicuspid aortic valves, coarctation of aorta, other valve abnormalities and septal defects. The participants in our study also had the same abnormalities other than aortic coarctation. Also there is a risk of hypertension, ischemic heart disease and stroke (Gravholt 2002). That shows the need for a screening echocardiogram when the diagnosis of TS is made. In the study population 19 (57.5%) patients were screened by echocardiography and among them 5 (15.2%) patients had cardiac abnormalities. Nobody had hypertension, Ischemic heart disease or stroke. But follow up is needed as they are still less than 30 years of age.

It is well known that although patients with TS have normal intelligence and that they have the problems with visual spatial skills (Hong *et al.* 2009). There were limitations to test IQ in the study population in a proper way as done in a psychiatric ward and mini mental state examination and executive function screen including clock drawing test were used to get an idea about the visual spatial skills and cognitive functions according to its scoring system. School performances, knowledge in mathematics, social history about the occupation and other capabilities were inquired to get an idea about their cognitive functions. According to the results among those who could perform the test 50% were cognitively normal.

From this study it can be concluded that the phenotype of the Sri Lankan TS patients is more or less similar to the common phenotype seen in other parts of the world.

4.4 Cytogenetic abnormalities and its Correlation with the clinical phenotype

This is the first study done in Sri Lanka to find the cytogenetic abnormalities and to correlate the clinical phenotype with the cytogenetic aetiology of the TS patients. If any correlation was found, it would be an added advantage to both patients and as well as to clinicians in the follow up of the TS patients. Knowing the common associations of the complications with the chromosomal abnormalities will help them to concentrate on those features in long term management. It will uplift the quality of their lives and the health status.

Considering the post natal features, all the features presented in the study group were seen in the patients with classical TS. All 3 patients who had dorsal oedema of the hands and feet, the only patient who had cystic hygroma and 75% who had extra skin around the neck were from the classical TS group. Epicanthal folds were found only in patients with classical TS but this finding was not found to be statistically significant.

Short stature being one of the characteristic features of TS showed no significant association in our study with the cytogenetic abnormalities (P value > 0.05) as it was a common feature of classical TS 12 (44.4%) and also of other TS variants 15 (55.6%). It is reported in the scientific literature that short stature is a typical feature that can be seen in almost all patients with classical TS and about 96% in mosaic TS and other TS variants (Elsheikh *et al.* 2002; Bondy 2007). Other frequent features such as webbed neck, high arched palate, epicanthal folds, hyper convex nails and wide carrying angle also had no significant association with the cytogenetic variation. They were common in patients regardless of the cytogenetic abnormality.

Primary amenorrhoea, ptosis, low posterior hair line, shield shaped chest and widely spaced nipples had a significant association with the cytogenetic variation in this study. Those features were more frequently associated with classical TS group than the other variants. When a suspected TS patient comes to a physician with the above features before doing a

karyotype he/she will be able to diagnose her as a classical TS patient with the help of the findings of this study. That fulfills one aim of our study.

In an Iranian study which was done to find out the effects of cytogenetic abnormalities on the phenotype of patients with TS, they concluded that there was a strong correlation between the cytogenetic abnormality and the phenotype and these clinical features are more severe in patients with classical TS than in the other variants (Nazarabadi 2005). Those findings are compatible with our study.

4.5 *PTPN11* mutations

As mentioned in the introduction NS is a main differential diagnosis for TS. Though there are multiple genes involved in the causation of NS, for the formation of clinical features which are common with TS such as short stature and webbed neck the mutations of the *PTPN11* gene are also reported to be responsible (Kosaki *et al.* 2002). In view of giving a diagnosis for the patients who come to the HGU with a clinical diagnosis of TS and receiving the karyotype as normal (46, XX), in our study the 4 exons were screened for mutations in a group of 24 patients. The patients were selected from the database and the records available at HGU from 2006 onwards. The exons (3, 8, 9 and 13) which were selected for screening were reported as hot spots in previous studies (Tartaglia M 2001; Kosaki *et al.* 2002; Tartaglia 2002; Loh *et al.* 2004).

As mentioned in the results only one silent mutation was found in exon 3 (c.255C>T) of *PTPN11* gene in patient number 45. It is reported as a polymorphic variant by Tartaglia, M. et al. (Tartaglia 2002). It causes no change in the Histidine protein i.e. causes no change in the phenotype too. But the patient who had the mutation was referred for karyotyping with a clinical diagnosis of TS and was short in stature. It is possible that she may have a mutation elsewhere in the gene. In patient number 2 a sequence variation was found in exon 9

(c.1052A>G) and in patient number 31 in the intervening sequence between exon 9 and 10 (c.934-27T>C) of *PTPN11* gene. These were not reported in scientific literature.

The reference sequences of both sequence variations were aligned with the tool BLAT in UCSC Genome Browser (hg19) and both neucleotides were found to be conserved across species. The 1st one (c.1052A>G) in all species including zebra fish while the 2nd one (c.934-27T>C) in human, rhesus, mouse, dog, elephant and opossum. It is believed that a mutation in a highly conserved region is more likely to be pathogenic (Sarah 2010). In this study also patient number 2 who had the mutation at c.1052A>G had a phenotype suggestive of both TS and NS with short stature, scoliosis and short and webbed neck. The patient number 31 also had features suggestive of both syndromes such as short and webbed neck, prominent ears, ptosis, scoliosis and wide carrying angle. Bioinformatics analysis done using PolyPhen (Polymorphism Phenotyping) version 2 of the mutation at c.1052A>G was predicted as benign. However it is a nonsynonymous SNP (single nucleotide polymorphism) that results in a substitution of the amino acid Arginine by Glutamine. So further investigations will be needed to detect the functional significance of these sequence variations.

5. CONCLUSION

Cytogenetic abnormalities in patients with TS in Sri Lanka were identified in this study. By studying the phenotypic pattern of this population, it was found that there was an association between the cytogenetic abnormalities and the phenotype of TS. The clinical features are more severe and common in the classical TS patients which is the most frequently found cytogenetic abnormality in Sri Lanka. According to this study primary amenorrhoea, ptosis, low posterior hair line, shield shaped chest and widely spaced nipples are the features mainly seen in patients with TS who have a cytogenetic abnormality.

Three sequence variations were found in the *PTPN11* gene of patients with clinical features of TS who have no cytogenetic abnormalities. Two of them need further investigations to determine whether they are causative.

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APPENDIX: Documents used for subject recruitment

This appendix contains the information sheets, consent forms which were used for subject recruitment in Sri Lanka and the data collection booklet.

- Information sheets for study participants used for the recruitment of patients with TS and the patients who have a clinical diagnosis of TS and a normal karyotype in Sinhala, English and Tamil languages.
- Consent forms used for recruitment of patients with TS and the patients who have a clinical diagnosis of TS and a normal karyotype in Sinhala, English and Tamil languages.
- The data collection booklet

තොරතුරු පත්‍රිකාව

ටර්නර් සහලක්ෂණ තත්ත්වය සහිත ලාංකීය රෝගීන්ගේ රෝග ලක්ෂණ වල විවිධත්වය සහ එම රෝග ලක්ෂණ සහ වර්ණදේහ අක්‍රමිකතා විවිධත්වය අතර සම්බන්ධය අධ්‍යයනය

කොළඹ වෛද්‍ය පීඨයේ මානව ප්‍රවේණි විද්‍යා අංශයේ පශ්චාත් උපාධියක් හදාරන වෛද්‍ය සුභාෂි කරුණාරත්න වන මම ඇතුළු අනෙකුත් පර්යේෂණ සාමාජිකයින් විසින් කරනු ලබන ඉහත සඳහන් පර්යේෂණයට සහභාගී වීම සඳහා ඔබ හට ආරාධනා කිරීමට අපි කැමැත්තෙමු.

අපගේ පර්යේෂණ කණ්ඩායමේ සාමාජිකයින් වනුයේ :

- මහාචාර්ය වජිර එච් . ඩබ්ලියු . දිසානායක (ප්‍රධාන අධීක්ෂක) - කොළඹ විශ්ව විද්‍යාලයේ වෛද්‍ය පීඨයේ කාය ව්‍යවච්ඡේද ඒකකයට සම්බන්ධ මහාචාර්ය වරයෙකි.
- මහාචාර්ය රොහාන් . ඩබ්ලියු . ජයසේකර (අධීක්ෂක) - කොළඹ විශ්ව විද්‍යාලයේ වෛද්‍ය පීඨයේ කාය ව්‍යවච්ඡේද ඒකකයට සම්බන්ධ මහාචාර්ය වරයෙකි.
- වෛද්‍ය සුභාෂි කරුණාරත්න (ප්‍රධාන පර්යේෂකවරිය) - කොළඹ විශ්ව විද්‍යාලයේ වෛද්‍ය පීඨයේ මානව ප්‍රවේණි විද්‍යා අංශයේ පශ්චාත් උපාධි අපේක්ෂිකාවකි.

1. මෙම අධ්‍යයනයේ අරමුණ

මෙම පර්යේෂණයේ ප්‍රධාන අරමුණ වනුයේ ටර්නර් සහලක්ෂණය සහිත ලාංකීය රෝගීන්ගේ රෝග ලක්ෂණ වල විවිධත්වය අධ්‍යයනය කිරීමත් , තවද එම රෝගීන්ගේ සායනික රෝග ලක්ෂණ සහ වර්ණදේහ විවිධත්වය අතර සම්බන්ධතාවයක් තිබේදැයි පරීක්ෂා කිරීමත්ය.

2. ස්වෙච්ඡා සහභාගීත්වය

මෙම අධ්‍යයනය සඳහා ඔබගේ සහභාගීත්වය ඔබගේ කැමැත්තෙන්ම සිදු කරන්නකි. මෙම අධ්‍යයනය සඳහා සහභාගී නොවීමට ඔබට පූර්ණ අයිතිය ඇති අතර සහභාගී වීමට කලින් කැමැත්ත ප්‍රකාශ කර තිබුනද ඕනෑම අවස්ථාවක අපහට දැනුම් දී

ඔබට අධ්‍යයනයෙන් ඉවත් වීමට පූර්ණ අයිතිය ඇත. එමගින් ඔබට කිසිදු බලපෑමක් ඇති නොවේ.

3. කාල සීමාව, පර්යේෂණයේ ක්‍රියා පිළිවෙල සහ සහභාගී වන්නන්ගේ වගකීම

මෙම පර්යේෂණයට සහභාගී වන්නට ඔබ කැමති නම් , ඔබගෙන් ප්‍රශ්න ඇසීමට , ඔබව පරීක්ෂා කිරීමට සහ ඔබගේ වෛද්‍ය වාර්තා පිරික්සීමට අපහට ඔබගේ අවසරය අවශ්‍ය වේ. තවද වර්ණදේහ පරීක්ෂණය සඳහා මි.ලී. 5 ක පමණ රුධිර සාම්පලයක් ඔබගෙන් අපට අවශ්‍ය වේ. නමුත් ඔබට ටර්නර් සහලක්ෂණ තත්ත්වය තිබෙන බව කලින් තහවුරු කර ඇත්නම් නැවත රුධිරය ලබා ගන්නේ නැත. අප විසින් කරනු ලබන පර්යේෂණයේ දත්ත , විද්‍යාත්මක සඟරාවක පල කිරීම සඳහාද ඔබගේ අවසරය අවශ්‍ය වේ. මෙම ප්‍රතිඵල පල කිරීමේදී ඔබගේ නම හෝ ඔබව හඳුනාගත හැකි අන්දමේ වෙනයම් තොරතුරක් හෝ අප විසින් සපයන්නේ නැත.

4. මින් ලද හැකි ප්‍රතිලාභ

මෙම පරීක්ෂණයට සහභාගී වීමෙන් ඔබට මෙම ටර්නර් සහලක්ෂණ තත්ත්වය ඇතිකිරීම සඳහා බලපාන වර්ණදේහ අක්‍රමිකතාව කුමක්දැයි දැනගත හැකිය. යම් හෙයකින් ඔබට ටර්නර් සහලක්ෂණය නොමැති නම් එම රෝග ලක්ෂණම ඇති කරවන නූනාන් සහලක්ෂණය නම් තත්ත්වය ඇති කරවන සුලබම ජානයක අක්‍රමිකතාවයක් ඔබට ඇතිදැයි අපි පරීක්ෂා කර බලන්නෙමු. ජානමය තත්ත්වය පිළිබඳව ලබා ගන්නා දැනුම මගින් ඔබගේ වෛද්‍යවරයාට , වඩා හොඳ අවබෝධයකින් ඔබගේ රෝගය පාලනය කිරීමට හැකියාවක් ලැබෙනු ඇත. එයට අමතරව මෙම පර්යේෂණයට සහභාගී වන රෝගියාට , අයගේ දෙමව්පියන්ට හෝ භාරකරුවන්ට මෙම රෝග තත්ත්වය සහ ඔබගේ ප්‍රතිඵල ගැන ජාන උපදේශනයක්ද අප ආයතනය මගින් නොමිලේ සපයනු ලැබේ. මෙම පර්යේෂණය , මෙම රෝගය ගැන ශ්‍රී ලංකාවේ රෝගීන් අතර දැනට පවතින දැනුම තවත් වැඩි කිරීමට උපකාරී වේ. ශ්‍රී ලංකාවේ රෝගීන්ගේ මෙම රෝගය ඇති කිරීමට බලපාන සුලබ වර්ණදේහ වර්ගීකරණය ගැන අප මින් ලබා ගන්නා දැනුම , අනාගතයේදී මෙම රෝගය වඩා පහසුවෙන් හඳුනා ගැනීම සඳහා අඩු මිල ජාන පරීක්ෂණ හඳුන්වා දීමට ඉමහත් උපකාරයක් වනු ඇත.

5. අවදානම්, අනතුරු සහ අපහසුතා

ටර්නර් සහලක්ෂණ තත්ත්වයෙහි වර්ණදේහමය විවිධත්වය පරීක්ෂා කිරීම සඳහා හෝ අවශ්‍ය වුවහොත් නූනාන් සහලක්ෂණ තත්වය ඇති කරවන සුලබ ජානයක අක්‍රමිකතාවය පරීක්ෂා කිරීම සඳහා රුධිර සාම්පලයක් ලබා ගන්නා අතර මෙම රුධිර සාම්පලය ලබා ගැනීමේදී ඔබට යම් අපහසුතාවයක් ඇති විය හැක. කලාතුරකින් රුධිර සාම්පලය ලබා ගැනීමේදී එන්නත් කටුව නිසා යම් තැල්මක් එම ස්ථානයේ හට ගත හැක. මෙම තත්ත්වයන් අවම කර ගැනීම සඳහා රුධිර සාම්පලය ලබා ගැනීම සියලුම ආරක්ෂිත තත්ත්වයන් යටතේ පළපුරුදු හෙද නිලධාරිනියක් මගින් සිදු කරනු ලැබේ.

6. දීමනා

ඔබ මෙම අධ්‍යයනයට සහභාගී වීම වෙනුවෙන් ඔබට දීමනාවක් නොලැබේ. එහෙත් ඔබට ඔබගේ රෝගයේ වර්ණදේහමය අක්‍රමිකතාවය පිළිබඳ වාර්තාවක් ලැබේ.

7. රහසිගත බව

සියලුම තොරතුරු සහිත වාර්තාවන් සහ අධ්‍යයනය මගින් ලබා ගන්නා දත්තයන්ගේ රහස්‍යභාවය තහවුරු කරන අතර , ඔබගේ අනන්‍යතාවය හඳුනාගත හැකි ආකාරයේ කිසිවක් ඔබගේ කැමැත්තකින් තොරව හෙළි කිරීමක් , ඉදිරිපත් කිරීමක් හෝ ප්‍රකාශයට පත් කිරීමක් සිදු කරනු නොලැබේ. දත්ත එකතු කිරීමේ පත්‍රිකාව සකසා ඇත්තේද ඔබගේ රහස්‍යභාවය තහවුරු කෙරෙන අයුරිනි. විද්‍යාත්මක සඟරාවක මෙම පර්යේෂණ වාර්තා පල කිරීමට අවශ්‍ය වූ විටද කිසිදු අයුරකින් ඔබගේ අනන්‍යතාවය හෙළි නොවෙන අයුරින් අපි එය පල කරන්නෙමු.

8. අධ්‍යයනයට සහභාගීවීම නැවත්වීම

අධ්‍යයනයට සහභාගීවීමට දුන් කැමැත්ත ඉවත් කර ගැනීම අධ්‍යයනයේ කුමන හෝ අදියරකදී සිදු කිරීමට ඔබට හැක. එසේ සිදු කරන්නේනම් එම තීරණය ගත් විගසම ඒ බව අපහට කරුණාකර දෙනුම් දෙන්න. නමුත් එකතු කර ගන්නා ලද දත්ත ප්‍රකාශයට පත් කිරීමෙන් පසුව ඔබට අධ්‍යයනයෙන් ඉවත් වීමට නොහැක.

9. වැඩිදුර තොරතුරු

ඔබට මේ ක්‍රියා පටිපාටීන් පිළිබඳව කිසියම් ප්‍රශ්නයක් ඇත්නම් හෝ වැඩිදුර තොරතුරු අවශ්‍ය නම් කරුණාකර පහත සඳහන් වෛද්‍යවරුන් අමතන්න.

මහාචාර්ය වජිර එච්. ඩබ්ලියු. දිසානායක - මානව ප්‍රවේණි විද්‍යා අංශය , වෛද්‍ය පීඨය , කොළඹ.

දු.ක. 112689545

වෛද්‍ය සුභාෂි කරුණාරත්න - පශ්චාත් උපාධි අපේක්ෂිකා, මානව ප්‍රවේණි විද්‍යා අංශය, වෛද්‍ය පීඨය , කොළඹ.

දු.ක. 112689545

INFORMATION SHEET

STUDY OF PHENOTYPIC VARIATIONS AND GENOTYPE – PHENOTYPE CORRELATION IN SRI LANKAN PATIENTS WITH THE TURNER SYNDROME PHENOTYPE

This study is conducted by me, Dr. Subhashi Karunaratne, M.Sc. student in Clinical Genetics currently attached to the Human Genetics Unit, Faculty of Medicine, University of Colombo. I would like to invite you to take part in the research study titled **"Study of phenotypic variations and genotype – phenotype correlation in Sri Lankan patients with the Turner syndrome phenotype"** conducted by myself under the supervision of Prof. Vajira H.W. Dissanayake and Prof. Rohan W. Jayasekara at the Human Genetics Unit, Faculty of Medicine, University of Colombo.

1. Purpose of the study

The purpose of this study is to identify the genetic variations in patients with TS and to correlate the clinical features with the genetic variations.

2. Voluntary participation

Your participation in this study is voluntary. You are free to not participate at all or to withdraw from the study at any time despite consenting to take part earlier. There will be no loss of medical care or any other available treatment for your illness or condition to which you are otherwise entitled. If you decide not to participate or withdraw from the study, you may do so at any time by informing us.

3. Duration, procedures of the study and participant's responsibilities

We require your permission to ask you questions examine you and have access to your medical records. We need to take 5mls of venous blood from you to do the genetic test. (Blood will not be taken from patients who already have a genetic diagnosis of Turner syndrome). You only have to visit us once during the research period to provide the clinical data and blood sample for the study. We also need your permission to publish the data collected in a scientific journal. We will not mention your name or any other identifiable information about you when we publish the results.

4. Potential Benefits

Participation in this study will help you to know the genetic defect that has made you develop Turner syndrome and if not whether you have the commonest genetic mutation causing Noonan syndrome. This will be a valuable opportunity as genetic testing for Turner syndrome and Noonan syndrome both are not freely available in Sri Lanka. Knowledge about the different genetic defects and genotype – phenotype correlation would enable the doctors caring for you to understand your clinical condition better and provide improved care. In addition, participants and/or parents/guardians will be provided genetic counseling about each participant's test results. This study will contribute to the increasing of existing knowledge about Turner syndrome in Sri Lankan patients.

5. Risks, Hazards and Discomforts

Blood will be drawn to detect the genetic defect causing Turner or Noonan syndrome. When taking blood, you will feel some discomfort due to the needle prick and after blood is drawn, rarely there can be bruising at the needle prick site. In order to ensure that these risks are minimized, blood drawing will be done by a trained phlebotomist under aseptic conditions.

6. Reimbursements

There will be no payment for participating in the study, but you will be given a copy of the cytogenetic or molecular genetic test results.

7. Confidentiality

Confidentiality of all records is guaranteed and no information by which you can be identified will be released or published. The data collection booklet is designed to ensure confidentiality of information gathered. The electronic database containing the data will have only the subject study number and the database and the computer containing the database would be password protected. If it becomes necessary to publish some of the results in scientific journals, we will make sure to publish the results protecting the identity of the subject. These data will never be used in such a way that you could be identified in any way in any public presentation or publication without your express permission.

8. Termination of Study Participation

You may withdraw your consent to participate in this study at any time, without any penalty or effect on medical care or loss of benefits. Please notify us as soon as you decide to withdraw your consent. However, it will not be possible for you to withdraw once the results are sent for publication or once the results are published.

9. Clarification

If you have any questions about any of the tests/procedures or information or need to clarify any doubts, please feel free to ask any of the persons listed below at the Human Genetics Unit by calling 11 2689 545.

Prof. Vajira H.W. Dissanayake – Medical Geneticist, Human Genetics Unit, Faculty of Medicine, University of Colombo.

Dr. Subhashi Karunaratne – M.Sc. student, Human Genetics Unit, Faculty of Medicine, University of Colombo.

தகவற் பத்திரிகை

சூழல் இயைபாக்க மாற்றங்களும் மரபுவகையும் பற்றிய ஆய்வு – சூழல் இயைபாக்க வகையின் ரேர்னரின் நோய்த் தோற்றப்பாடுகளுடன் இலங்கை நோயாளிகள் கொண்டுள்ள பரஸ்பர சூழல் இயைபாக்கத் தொடர்பு.

இந்த ஆய்வானது, கொழும்பு பல்கலைக்கழ மருத்துவ பீடத்தின் மானிட மரபுப் பிரிவிலே (Human Genetics Unit) தற்போது இணைந்துகொண்டு, மருத்துவ மரபியலிலே (Clinical Genetics) முதுமாணி (M.Sc) மாணவராக இருக்கும் டாக்டர் சுபாஷி கருனாரட்ன (Dr. Subhashi Karunaratne) ஆகிய என்னாலே மேற்கொள்ளப்படுகிறது. கொழும்பு பல்கலைக்கழக மருத்துவ பீடத்தின் மானிட மரபுப் பிரிவின் பேராசிரியர் வஜிர எச்.டபிளியூ. திஸாநாயக்க (Prof. Vajira H.W. Dissanayake) என்பவரினதும் பேராசிரியர் றொஹான் டபிளியூ ஜயசேகர (Prof. Rohan W. Jayasekara) என்பவரினதும் மேற்பார்வையின் கீழ் "*சூழல் இயைபாக்க மாற்றங்களும் மரபுவகையும் பற்றிய ஆய்வு – சூழல் இயைபாக்க வகையின் ரேர்னரின் நோய்த்தோற்றப்பாடுகளுடன் இலங்கை நோயாளிகள் கொண்டுள்ள பரஸ்பர சூழல் இயைபாக்கத் தொடர்பு*" என்ற தலைப்பிலான ஆய்விலே பங்கேற்கும்படியாக நான் உங்களுக்கு அழைப்பு விடுக்க விரும்புகிறேன்.

1. ஆய்வின் நோக்கம்

இந்த ஆய்வின் நோக்கமானது TS உள்ள நோயாளிகளின் மரபு மாற்றங்களை இனங்கண்டு, அதனை மரபியல் மாற்றங்களுடன் தொடர்புபட்ட மருத்துவ அம்சங்களுடன் தொடர்புபடுத்துவதாகும்.

2. தன்னார்வப் பங்கேற்பு

இந்த ஆய்விலே உங்களது பங்கேற்பு தன்னார்வரீதியானதாகும். இதிலே நீங்கள் முற்றாகப் பங்கேற்காதிருக்கவோ அல்லது முன்னதாகப் பங்கேற்கச் சம்மதித்து, எந்த ஒரு வேளையிலும் அதிலிருந்து வாபஸ் பெறுவதற்கோ சுயாதீனம் உங்களுக்கு உண்டு. உங்களது மருத்துவப் பராமரிப்புக்கோ அல்லது உங்களது நோய்க்கு அல்லது நிலைமைக்கென உங்களுக்கு உரித்தான எந்த ஒரு மருத்துவச் சிகிச்சைக்கோ எவ்வித இழப்பும் ஏற்படாது. இதிலே நீங்கள் முற்றாகப் பங்கேற்காதிருக்கவோ அல்லது பங்கேற்கச் சம்மதித்து,

எந்த ஒரு வேளையிலும் அதிலிருந்து வாபஸ் பெறுவதற்கோ தீர்மானித்தால் எவ்வேளையிலும் அதனை எங்களுக்கு அறிவித்து நீங்கள் அவ்விதம் செயற்படலாம்.

3. கால அவகாசம், ஆய்வின் செயன்முறைகள் மற்றும் பங்கேற்பாளரின் பொறுப்புக்கள்.

உங்களிடம் கேள்விகள் கேட்பதற்கும், உங்களைச் சோதித்திடவும், உங்களது மருந்துவப் பதிவுகளுக்கான பெற்றடைவுக்கும் உங்களது அனுமதியினை நாம் கோருகிறோம். மேலும் உங்களது மரபுப் பரிசோதனையை மேற்கொள்வதற்கென உங்களது நாடி இரத்தம் 5 மி.லீ. எமக்குத் தேவைப்படும். (ரேர்னர் நோய்த்தோற்றப்பாட்டின் மரபியல் நோய்க்கண்டுபிடிப்பை ஏற்கெனவே கொண்டுள்ள நோயாளிகளிடம் இருந்து இரத்தம் பெறப்படமாட்டாது). ஆய்வுக் காலத்தின்போது நீங்கள் ஒருமுறை மாத்திரமே எம்மிடம் வந்து, ஆய்வுக்கு வேண்டிய இரத்தத்தையும் உங்களது மருத்துவத் தரவுகளையும் வழங்கவேண்டியதாய் இருக்கும். நாம் அப்படியாகச் சேகரித்த தரவுகளை மருத்துவ ஏட்டிலே பதிப்பிப்பதற்கு உங்களது அனுமதியும் எமக்குத் தேவை. அப்படியாக எமது பெறுதிகளை நாம் பதிப்பிக்கும்போது உங்களது பெயரையோ அல்லது உங்களை இனங்காட்டும் எந்த ஒரு தகவலையுமோ நாம் குறிப்பிடமாட்டோம்.

4. இதிலே உள்ள நன்மைகள்

இந்த ஆய்விலே கலந்துகொள்ளும்போது, உங்களிலே ரேர்னர் நோய்த்தோற்றப்பாட்டினை விருத்தியாக்கியுள்ள மரபுவழிக் குறைபாடு எது என்பதை நீங்கள் அறிந்து கொள்ள உதவும். அப்படி அல்லாவிட்டால் நூனான் (Noonan) நோய்த் தோற்றப்பாடுகளுக்குக் காரணமாய் அமைந்துள்ள பொதுவான இயையுமாற்றம் உங்களுக்கு உள்ளதா இல்லையா என்பதை நீங்கள் அறிந்துகொள்ளலாம். இது பெறுமதிவாய்ந்த ஒரு சந்தர்ப்பமாகும். ஏனெனில், ரேர்னர் நோய்த்தோற்றப்பாடுகளுக்கோ அல்லது நூனான் நோய்த்தோற்றப்பாடுகளுக்கே உரிய மரபுவழிப்பரிசோதனை லங்கையிலே இனாமாகக் கிடைக்காது. பல்வேறு மரபுக் குறைபாடுகளையும் மரபுவழிவகையையும் பற்றிய அறிவு – அதன் சூழல் இயைபாக்கத் தொடர்புறவு, உங்களைப் பராமரிக்கும் மருத்துவர்களுக்கு உங்களது மருத்துவ நிலைபரத்தை நன்றாகப் புரிந்துகொள்ளவும், முன்னேற்றப்பட்ட பராமரிப்பை அவர்கள் உங்களுக்கு வழங்கவும் வழிவகுத்திடும். மேலும் பங்கேற்பாளர்கள் மற்றும்/அல்லது பெற்றோர்/ பராமரிப்பாளர்களுக்கு ஒவ்வொரு பங்கேற்பாளர்களினதும் பெறுதிகளையிட்ட மரபுவழி ஆலோசனைவழங்கல் (genetic counseling) வழங்கப்படும். இந்த ஆய்வானது இலங்கை நோயாளிகளில் உள்ள ரேர்னர் நோய்த்தோற்றப்பாடு பற்றிய அறிவை அதிகரித்திட வகைசெய்திடும்.

5. இதில் பொதிந்துள்ள ஆபத்துக்களும், இடராபத்துக்களும், அசௌகரியங்களும்.

ரேர்னர் அல்லது நூனன் நோய்த் தோற்றப்பாடுகளுக்குக் காரணமாயுள்ள மரபுக் குறைபாட்டினைக் கண்டறிவதற்கு இரத்தம் பெறப்படும். அப்படியாக இரத்தம் எடுப்பதற்கு ஊசி குத்தப்படும்போதும், அதற்பின்பும் உங்களுக்கு சில அசௌகரியங்கள் உணரப்படலாம். ஊசி குத்தப்பட்ட இடத்திலே சிலவேளைகளிலே சிறு காயம் ஏற்படலாம். இத்தகைய ஆபத்துக்களைக் குறைக்கும்படிக்கு, உங்களது இரத்தம் நோய்க்கிருமிகளற்ற நிலைமயிலே குருதி பெறுவதற்கு நன்கு பயிற்றப்பட்ட குருதிவடிப்பு மருத்துவ வல்லுனரால் (phlebotomist) மேற்கொள்ளப்படும்.

6. மீளளிப்புக்கள்

இந்த ஆய்விலே பங்கேற்பதற்கு எவ்வித கொடுப்பனவும் வழங்கப்படமாட்டாது. ஆயினும் மூலக்கூற்று மரபணு அல்லது மூலக்கூற்று மரபுவழிச் சோதனையின் பெறுபேறுகளின் பிரதியொன்று உங்களுக்கு வழங்கப்படும்.

7. இரகசியங்காத்தல்

சகல பதிகவுகளும் இரகசியமாகப் பேணப்படுவதற்கு உத்தரவாதம் வழங்குகிறோம். உங்களை இனங்காணக்கூடியதான எவ்வித தகவலும் பதிப்பிக்கப்படமாட்டாது. தரவுகளைச் சேகரிக்கும் குறுநூல் சேகரிக்கப்பட்ட தகவல்களையிட்ட இரகசியம் காப்பதை உறுதிப்படுத்தும் விதத்திலேயே வடிவமைக்கப் பட்டுள்ளது. தரவுகளைக் கொண்டுள்ள மின்னிலத்திரனியல் தகவற்தளம் ஆக ஆய்வுப்பொருளின் இலக்கத்தையும், தகவற் தளத்தையும் மட்டுமே கொண்டிருக்கும். அத்துடன் இத் தகவற்தளத்தைக் கொண்டுள்ள கணனி இரகசியச்சொல்லினால் பாதுகாக்கப்பட்டிருக்கும். விஞ்ஞான ஏடுகளிலே சில பெறுதிகளைப் பதிப்பிக்கவேண்டிய அவசியம் ஏற்படுமேயாயின், ஆய்வுப்பொருளின் அடையாளப்படுத்துதலைப் பாதுகாத்திடும் விதத்திலே நாம் பெறுதிகள் பதிப்பிக்கப்படுவதை உறுதிசெய்வோம். எந்த வெகுஜன சமர்ப்பிப்புக்களிலே அல்லது பதிப்புக்களிலோ உங்களது அனுமதி பெறப்படாமல் இத் தகவல்கள் உங்களை எந்த வகையிலும் அடையாளங் கண்டுகொள்ளும் விதத்திலே பயன்படுத்தப்படமாட்டாது.

8. ஆய்விலே பங்கேற்பு முடிவுபெறுதல்

இந்த ஆய்வில் இருந்து உங்களது இணக்கப்பாட்டினை எவ்வேளையிலும், எந்த ஒரு தண்டமுமோ அல்லது உங்களுக்கான மருத்துவப் பரமாமரிப்போ அல்லது நன்மைகளிலோ எவ்வித இழப்பும் இன்றி நீங்கள் வாபஸ்

பெற்றுக் கொள்ளலாம். தயவுசெய்து அப்படியாக வாபஸ்பெற நீங்கள் தீர்மானித்தால் அதனை விரைவாக எமக்கு அறியத்தாருங்கள். ஆயினும், இந்த ஆய்வின் பெறுதிகள் பதிப்பிக்கப்படுவதற்காக அனுப்பப்பட்டபின்போ அல்லது அவை பதிப்பிக்கப்பட்டபின்போ இதில் இருந்து நீங்கள் வாபஸ் பெற்றுக்கொள்வது சாத்தியமாகாது.

9. தெளிவுபடுத்துதல்கள்

இந்தப் பரிசோதனைகள் / செயன்முறைகள் பற்றி உங்களுக்கு ஏதாவது கேள்விகள் இருக்குமேயானால் அல்லது தகவல்கள் தேவைப்படுமேயானால் அல்லது உங்களது ஐயங்களை நிவர்த்திசெய்துகொள்ள வேண்டுமேயானால், மனித மரபுப் பிரிவிலே கீழே குறிப்பிடப்பட்டவர்களை 11 2689545 எனும் தொலைபேசியிலே தொடர்புகொண்டு கேள்விகளைக் கேட்கத் தயங்க வேண்டாம்.

பேராசிரியர் வஜிர எச்.டபிளியூ. திஸாநாயக்க (Prof. Vajira H.W. Dissanayake) – மருத்துவ மரபுநிபுணர் (Medical Geneticist), மானிட மரபுப் பிரிவு, மருத்துவப் பீடம், கொழும்பு பல்கலைக்கழகம்.

டாக்டர் சுபாஷி கருனாரட்ன (Dr. Subhashi Karunaratne), முதுமாணி (M.Sc) மாணவர், மானிட மரபுப் பிரிவு (Human Genetics Unit), மருத்துவபீடம், கொழும்பு பல்கலைக்கழகம்

කැමැත්ත ප්‍රකාශ කිරීමේ පත්‍රය

ටර්නර් සහලක්ෂණ තත්වය සහිත ලාංකීය රෝගීන්ගේ රෝග ලක්ෂණ
සහ වර්ණදේහමය විවිධත්වයෙහි අධ්‍යයනය

(a) සහභාගී වන්නන් විසින් පිරවීම සඳහාය.

මෙම පත්‍රය සහභාගී වන්නන් /භාරකරුවන් විසින් සම්පූර්ණයෙන් පිරවිය යුතුය.

1. අධ්‍යයනය සම්බන්ධයෙන් තොරතුරු පත්‍රිකාවේ පැහැදිලි කරන ලද කරුණු ඔබට තේරුණාද? (කරුණාකර තොරතුරු පත්‍රිකාවේ පිටපතක් ඔබ ලබාගන්න)

ඔව්/නැහැ

2. මෙම අධ්‍යයනය සම්බන්ධව සාකච්ඡා කිරීමට හා ඒ පිළිබඳව ප්‍රශ්න ඇසීමට ඔබට අවස්ථාවක් ලැබුණා ද?

ඔව්/නැහැ

3. ඔබ ඇසූ ප්‍රශ්න සියල්ලටම සෑහීමකට පත්විය හැකි පිළිතුරු ලැබුණාද?

ඔව්/නැහැ

4. මෙම අධ්‍යයනය සම්බන්ධයෙන් ප්‍රමාණවත් තොරතුරු ලැබුණාද? **ඔව්/නැහැ**

5. මෙම අධ්‍යයනය සම්බන්ධයෙන් ඔබට පැහැදිලි කරන ලද්දේ කවුරුන් විසින්ද?

……

6. කිසිදු කරුණු දැක්වීමකින් තොරව, මෙම අධ්‍යයනයෙන් ඉවත් වීමට ඔබ හට ඕනෑම අවස්ථාවක හැකියාව ඇති බව සහ එය ඔබගේ ඉදිරි වෛද්‍ය ප්‍රතිකාර සඳහා බලනොපාන බව පැහැදිලි වූවාද?

ඔව්/නැහැ

7. ඔබේ වෛද්‍ය වාර්තා සහ පර්යේෂණ දත්ත මෙම අධ්‍යයනය සම්බන්ධ සාමාජිකයින් විසින් අධ්‍යයනය කෙරේ. සියලු වාර්තා සහ දත්තවල රහස්‍යභාවය තහවුරු කෙරේ. මෙම අධ්‍යයනය සම්බන්ධ සාමාජිකයින්ට තොරතුරු ලබා දීමට එකඟ වෙනවාද? **ඔව්/නැහැ**

8. මෙම අධ්‍යයනයෙන් පසුව ඉතිරි වන රුධිර සාම්පලයක් ඇතොත් ඉදිරියේදී කෙරෙන රෝගය සම්බන්ධ පර්යේෂණ සඳහා භාවිතා කිරීමට ඔබ එකඟ වෙනවාද?

ඔව්/නැහැ

9. රුධිර සාම්පල පිටරට යැවීමට එකඟ වෙනවාද?

ඔව්/නැහැ

10. මෙම අධ්‍යයනයට සහභාගී වීම සම්බන්ධයෙන් තීරණයකට එලඹීමට ඔබට

ඇති තරම් කාලය ලැබුණා ද?

ඔව්/නැහැ

11. ඔබ මෙම අධ්‍යයනයට සහභාගී වීමට එකඟ වෙනවාද? **ඔව්/නැහැ**

සහභාගී වන්නන් /භාරකරුවන්

අත්සන:..... දිනය:.....

නම:.....

(b) පර්යේෂක විසින් පිරවීම සඳහාය.

මෙම අධ්‍යයනය සම්බන්ධ කරුණු, මා විසින් අධ්‍යයනයට ස්වේච්ඡාවෙන් සහභාගී වන්නන් හට පැහැදිලි කරන ලදී. ඔහු/ඇය විසින් මෙම අධ්‍යයනයට සහභාගී වීමට කැමැත්ත ප්‍රකාශ කරන ලදී.

පර්යේෂකගේ අත්සන:..... දිනය:.....

නම:.....

CONSENT FORM

STUDY OF PHENOTYPIC VARIATIONS AND GENOTYPE – PHENOTYPE CORRELATION IN SRI LANKAN PATIENTS WITH THE TURNER SYNDROME PHENOTYPE

A. To be completed by the participant/guardian

The participant/guardian should complete the whole of this sheet by himself/herself.

1. Have you read the information sheet? (Please keep a copy for yourself) YES/NO

2. Have you had an opportunity to discuss this study and ask any questions? YES/NO

3. Have you had satisfactory answers to all your questions? YES/NO

4. Have you received enough information about the study? YES/NO

5. Who explained the study to you? ……

6. Do you understand that you are free to withdraw from the study at any time, without having to give a reason and without affecting your medical care? YES/NO

7. Sections of your medical notes, including those held by the investigators relating to your participation in this study may be examined by other research assistants. All personal details will be treated as STRICTLY CONFIDENTIAL. Do you give your permission for these individuals to have access to your records? YES/NO

8. Do you agree for your blood samples to be sent abroad? YES/NO

9. Do you agree to have left over blood samples and DNA be stored for future research into Turner syndrome under the supervision of the supervisor? YES/NO

10. Have you had sufficient time to come to your decision? YES/NO

11. Do you agree to take part in this study? YES/NO

Participant's/Guardian's

Signature…….....… Date…….……...

Name (BLOCK CAPITALS)……

…….....

B. To be completed by the investigator

I have explained the study to the above volunteer and she has indicated her willingness to take part.

Signature of investigator……...…….. Date……..……..

Name (BLOCK CAPITALS)…….…

……

இணக்கப்படிவம்

சூழல் இயைபாக்க மாற்றங்களும் மரபுவகையும் பற்றிய ஆய்வு – சூழல் இயைபாக்க வகையின் ரேர்னரின் நோய்த் தோற்றப்பாடுகளுடன் இலங்கை நோயாளிகள் கொண்டுள்ள பரஸ்பர சூழல் இயைபாக்கத் தொடர்பு.

பங்கேற்பாளரால் / பராமரிப்பாளரால் பூர்த்தியாக்கப்படவேண்டிய பகுதி

பங்கேற்பாளரால் / பராமரிப்பாளர் இந்தப் பகுதியை அவராகவே முழுமையாகப் பூர்த்திசெய்யவேண்டும்

1. இந்தத் தகவற்பிரதியை நீங்கள் வாசித்தீர்களா? (தயவுசெய்து இதன் பிரதியொன்றை நீங்கள் வைத்திருங்கள்)

ஆம் / இல்லை

2. இந்த ஆய்வைப்பற்றிக் கலந்துரையாடவோ அல்லது கேள்விகள் கேட்கவோ உங்களுக்குச் சந்தர்ப்பம் ஏதாவது வாய்த்ததா? ஆம் / இல்லை

3. எல்லாக் கேள்விகளுக்கும் உங்களுக்குத் திருப்திகரமான பதில்கள் கிடைத்ததா? ஆம் / இல்லை

4. இந்த ஆய்வைப்பற்றி போதிய தகவல்களை நீங்கள் பெற்றுக்கொண்டிருக்கிறீர்களா? ஆம் / இல்லை

5. இந்த ஆய்வினைப்பற்றி யார் உங்களுக்கு விளக்கிக்கூறியிருக்கிறார்கள்? .....

6. எந்தவித விளக்கமும் தரத் தேவை இல்லாமல், உங்களது மருத்துவப் பராமரிப்பையும் பாதிக்காமல் இந்த ஆய்வில் இருந்து எந்த வேளையிலும் வாபஸ் பெறுவதற்கு உங்களுக்குச் சுயாதீனம் உண்டு என்பதை நீங்கள் புரிந்துகொண்டிருக்கிறீர்களா? ஆம் / இல்லை

7. இந்த ஆய்விலே உங்களது பங்கேற்பு தொடர்பாக குறித்த ஆய்வாளர்களிடம் உள்ளவை உள்ளடங்கலாக, உங்களது மருத்துவக் குறிப்புக்களின் பகுதிகள் வேறு ஆய்வு உதவியாளர்களாலே பரிசோதிக்கப்படலாம். எல்லாப் பிரத்தியேகத் தகவல்களும் கட்டாயமாக இரகசியம் காக்கப்படும். உங்களது பதிவுகளுக்கான பெற்றடைவுகளுக்கான அனுமதியை இந்தத் தனிநபர்களுக்கு நீங்கள் வழங்குகிறீர்களா? ஆம் / இல்லை

8. உங்களது குருதி மாதிரிகள் மேல்நாடுகளுக்கு அனுப்பப்படுவதற்கு நீங்கள் இணங்குகிறீர்களா?

ஆம் / இல்லை

9. பெறப்பட்ட குருதியிலே எஞ்சியுள்ள பகுதியும் அதன் DNA உம் இந்த மேற்பார்வையாளரின் மேற்பார்வையின்கீழ் எதிர்கால ரேர்னர் நோய்த்தோற்றப்பாட்டு ஆய்வுகளுக்கெனச் சேமித்துவைக்கப்படுவதற்கு நீங்கள் அனுமதி வழங்குகிறீர்களா? ஆம் / இல்லை

10. உங்களது தீர்மானத்துக்கு வருவதற்கு உங்களுக்குப் போதிய நேர அவகாசம் இருந்ததா?

ஆம் / இல்லை

11. இந்த ஆய்விலே பங்கேற்பதற்கு நீங்கள் இணங்குகிறீர்களா?

ஆம் / இல்லை

…… ……..

பங்கேற்பாளர் / பராமரிப்பாளர் கையொப்பம் திகதி

பெயர் (தனித்த எழுத்துக்களில்): ……..

விசாரணை செய்பவரால் பூர்த்தியாக்கப்படவேண்டிய பகுதி

இந்த ஆய்வினை நான் மேற்படியான தன்னார்வத் தொண்டருக்கு விளக்கியிருக்கிறேன். அவர் இதிலே பங்கேற்பதற்குத் தனது இணக்கத்தைச் சுட்டிக்காட்டியிருக்கிறார்.

…… ……..

விசாரணை செய்பவரின் கையொப்பம் திகதி

பெயர் (தனித்த எழுத்துக்களில்)……..

STUDY OF PHENOTYPIC VARIATIONS AND GENOTYPE – PHENOTYPE CORRELATION IN SRI LANKAN PATIENTS WITH THE TURNER SYNDROME PHENOTYPE

PHENOTYPIC DATA

Subject Study Number | | | | | |

Date ……/……/……

Name of the Subject ……

Date of Birth …… /……/…….

Address ……

……

Telephone Number (Home) …… **Mobile** ……..

E-mail ……..

Referring Physician ……

Hospital ……

Ward No ……

Clinic No / BHT No ……..

Data Protection and Confidentiality

After completion of this page, ensure that the subject study number is entered on all pages of this booklet. Then detach this page and store separately from the remainder of the booklet.

Subject Study Number

Date of entry to the study

		–			–				

Date of Birth

		–			–				

Indication for karyotype analysis

CLINICAL HISTORY

Ante natal history

Ante natal USS (If available)

Cardiac defects

Cystic hygroma

Horse shoe kidney

Birth History

Birth weight	
Length	
Head Circumference	
Puffiness of the hands and feet	
Extra skin at the neck	
Cystic hygroma	
Hip dislocation	
Other	

Subject Study Number

Past medical history

Frequent middle ear Infections	
Frequent UTI	
Impaired vision	
Increased bone fractures	
Diabetes Mellitus	
Hypothyroidism	
Gastrointestinal disorders	

Past surgical history

Cardiac surgery

Other

Menstrual history

Attained Menarche	Yes	
	No	
Age of menarche		
Cycles	regular	
	Irregular	

Subject Study Number

Gynaecological history

Secondary amenorrhoea

Subfertility

Obstetric history

Parity

Assisted

Spontaneous

Family history and pedigree

(Indicating family history of short stature, primary amenorrhoea, secondary amenorrhoea, subfertility)

Consanguinity Yes/No
I
II
III
IV
V

Subject study number

Additional pedigree information

Location in pedigree	Clinical or other information

CLINICAL EXAMINATION

General examination

Height/Length	cm
Weight	Kg

Subject study number

Short neck	
Webbed neck	
Low posterior hair line	
Low set ears	
High arched palate	
Dental crowding	
Malocclusion	
Cataract	
Ptosis	
strabismus	
Epicanthal folds	
Red-green colour blindness	
Shield shaped chest	
Widely spaced nipples	
Nails - hypo plastic	
hyper convex	
Short fingers and toes	
Short fourth metatarsals	
Madelung deformity	
Increased carrying angle	
Multiple pigmented nevi	
Scoliosis	
Kyphosis	
Other	

Subject study number

Breast and pubic hair development according to Tanner Stage (refer appendix 1)

Breast Development (B)

Pubic hair development (PH)

B I		PH I	
B II		PH II	
B III		PH III	
B IV		PH IV	
B V		PH V	

Cognitive functions

Visual spatial skills

Hearing

Subject study number

Vision

Cardiovascular system

Blood pressure- mmHg

Murmurs

Abdomen

Subject study number

INVESTIGATIONS

Serum Hormone Levels

	Normal	High	Low	Not done
Serum LH Levels				
Serum FSH Levels				
Serum Oestrogen Levels				
Serum Testosterone				

USS –Abdomen and pelvis

Uterus Ovaries Kidneys

Laparoscopy findings

Uterus Ovaries Kidneys

Subject study number

Blood sugar

	Normal	Low	High	Not done
FBS/RBS				

Thyroid profile

	Normal	Low	High	Not done
TSH level				
Free T3 level				
Free T4 level				

Antithyroid antibodies

Normal	Low	High	Not done

ECG

Prolonged QT interval Sinus tachycardia Left bundle branch block

Echocardiography

Aortic valve changes Aneurysm formation Coarctation of aorta

Subject study number

CT scan of chest

Coarctation of aorta

Karyotype (If available)

Date:……../……./……
Findings:

TREATMENT

Oestrogen therapy

Progesterone therapy

Growth hormone therapy

Subject study number

Sampling record

Date of collection

		/			/				

	Label	volume	storage	comments
Na / Heparin				
K / EDTA				

Comments

Record reasons for any missing data and any additional relevant comments. **ENSURE THAT ANONYMITY IS PRESERVED.**

The booklet should be signed when ALL available data have been entered and cross checked with relevant data recorded elsewhere in this booklet.

Signed……. **Date……**

Investigator/Research Assistant

Subject study number

Appendix 1

Females - breast development

Tanner I

no glandular tissue: areola follows the skin contours of the chest (prepubertal) [typically age 10 and younger]

Tanner II

Breast bud forms, with small area of surrounding glandular tissue; areola begins to widen [10-11.5]

Tanner III

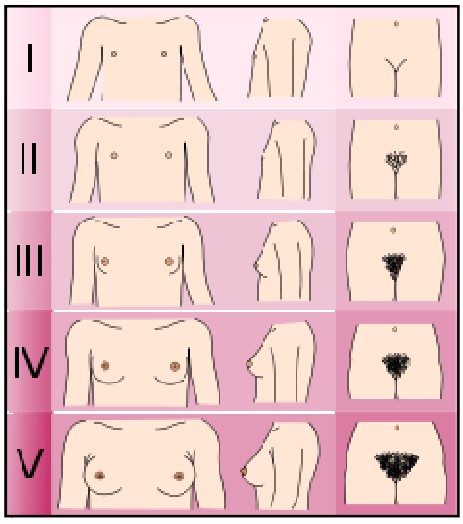
Breast begins to become more elevated, and extends beyond the borders of the areola, which continues to widen but remains in contour with surrounding breast [11.5-13]

Tanner IV

Increased breast size and elevation; areola and papilla form a secondary mound projecting from the contour of the surrounding breast [13-15]

Tanner V

Breast reaches final adult size; areola returns to contour of the surrounding breast, with a projecting central papilla area. [15+]



Subject study number | | | | | |

Appendix 2

MINI MENTAL STATE EXAMINATION (MMSE)

ORIENTATION

	SCORE (Circle one)
What is the year?	0 1
What is the season? (allow for error if beginning/end of season)	0 1
What month is it now? (allow for error if first/last day of month)	0 1
What day of the week is it today? (allow for error if near midnight)	0 1
What is today's date?	0 1
What country are we in now?	
What city/town are we in? (if rural, ask what province)	0 1
What suburb are we in? (if rural, ask what area/district)	0 1
What building/place are we in? (name or type)	0 1
What Ward/floor/room of the building/place are we in?	0 1
Score 1 point for correct response, maximum 10.	0 1

REGISTRATION

Listen carefully. I'm going to say three words.
You say them back when I've finished. Ready? (present 1 second each):

Read this way → *tick which items used*

1st trial	APPLE	COIN	CHAIR	☐
re-test	BALL	CAR	MAN	☐
2nd re-test	SHOE	FLAG	TREE	☐

0 1 2 3

Repeat up to 5 times (until patient can repeat all 3), but score only first trial.
Number of trials: .....

Now keep those words in mind. I'm going to ask you to say them again in a few minutes.

ATTENTION AND CALCULATION [Serial 7s]

Now I'd like you to subtract 7 from 100.
Then keep subtracting 7 from each answer until I tell you to stop.
What is 100 take away 7? (If needed say, keep going). 0 1
Record responses: ___ ___ ___ ___ ___ 0 1 0 1 0 1
(Score 1 point for each answer that is 7 less than the previous number. Maximum 5)
Don't remind patient of where they are up to. If they lose track, say something like, "Give your best guess."

If patient refuses to perform serial 7's or is clearly unable to, substitute WORLD item below.
Spell WORLD forward (correct any misspelling), then backward
(score backward spelling - 1 point for each letter that appears in the correct order)

RECALL

What were those three words I asked you to remember?

1	2	3

0 1 2 3

Score 1 point for each correct item, maximum 3.
Do not prompt but if no response, say "Take a moment" or "Take a guess".

Name: **Date:** **Examiner:**

SCORE
(Circle one)

NAMING

What is this? (Point to a pen or pencil) 0 1

What is this? (Point to a watch) 0 1

Alternative common objects (e.g., glasses, chair, and keys) can be used.
1 point each for correct name of whole, or part of, objects.

REPETITION

Listen carefully, I am going to ask you to repeat what I say. Ready?
"THE PASTRY COOK WAS ELATED." Now you say that. 0 1

Alternative item:
"NO IFS, ANDS, OR BUTS."
Repeat up to 5 times, but score only first trial. Number of trials: .....

COMPREHENSION

Listen carefully because I'm going to ask you to do something. (present piece of paper to patient's midline ONLY after giving complete instruction)

Take this piece of paper in your right/left *(non dominant)* **hand,** 0 1
fold it in half, 0 1
and put it on the floor 0 1

READING

Please read this and do what it says
(use separate page) 0 1

CLOSE YOUR EYES

WRITING

Please write me a sentence 0 1

(use separate page)
If person doesn't respond, say, "Write about the weather." (Cue used: yes/no)
The sentence must contain a subject, verb and make sense. Ignore grammar or spelling.

DRAWING

Please copy this design. (use separate page) 0 1
Score 1 point if drawing consists of two 5-sided figures that intersect to form a 4-sided figure.

TOTAL SCORE

30

Updated November 2004

EXECUTIVE FUNCTION SCREEN

CLOCK FACE
use back of separate page)
Draw a clock face. Put all of the numbers where they belong.
When patient completes this),
Now set the hands to 10 past 11.

Write notes in space below. Comment on time taken, behavioural observations e.g., did patient look confused), how they approached task (e.g., planning) etc.

COPY AND CONTINUE ("MW" pattern)
use separate page)
Copy this pattern underneath and continue the pattern until I say stop.

Write observation notes in space below.

SIMILARITIES
I am going to read two words to you, and I want you to tell me how they are alike or similar.

	2-point	1-point	0-point
In what way are a shirt and trousers alike? If 2-point answer not given say, "What category are they from?" *Tick if prompt given* ☐ Provide 2-point response if required. Don't prompt from here on.	clothing	material	Both brown
In what way are a carrot and a potato alike?	vegetable	food	Both can be peeled
In what way are a plate and a cup alike?	Crockery/ Eating utensils	Made from same material	Both go on table/ Both white
In what way are tennis and rugby alike?	sport	exercise	Both fun
In what way are a train and a motorbike alike?	transport	wheels	Make noise

Abstract ◄——► *Concrete*

WORD GENERATION

(use separate page)

I am going to say a letter of the alphabet. Then I want you to give me as many words that begin with that letter as quickly as you can. For example, if I say "S" you might give me "sit", "soft" or "simple". I do not want you to give words that are names of people or places such as "Silverdale" or "Sam". I don't want you to give words that are numbers such as "seven". Also do not use the same word again with different endings such as "sit", "sits" and "sitting".

So no names of people or places (pause), no words that are numbers (pause) and no words with different endings. Do you have any questions?
Begin when I say the letter. The first letter is "T". Go ahead.

Begin timing immediately. Allow 60 seconds for each letter, making a note of the 30-second mark.
Write down the actual words in the order in which they are produced. Mark repeats and violations (don't score these)
If patient discontinues before the end of 1 minute, encourage them to think of more words. If there is a silence of 15 seconds, repeat the basic instructions and the letter.

T	R	D

Total acceptable words generated	Total >20	15-19	<15
	normal	Mild to moderate impairment	More severe impairment

INITIATION AND IMPULSE CONTROL

Take my hand gently. (Take patient's dominant hand as though shaking hands).

If I say "red" squeeze my hand like this (demonstrate a quick, light squeeze)

If I say "green", do nothing.

Allow patient to practice a few times giving R / G in random order at a rate of about 1 per second.

Suggested sequence:
R R G R G R G G G R G R R G R

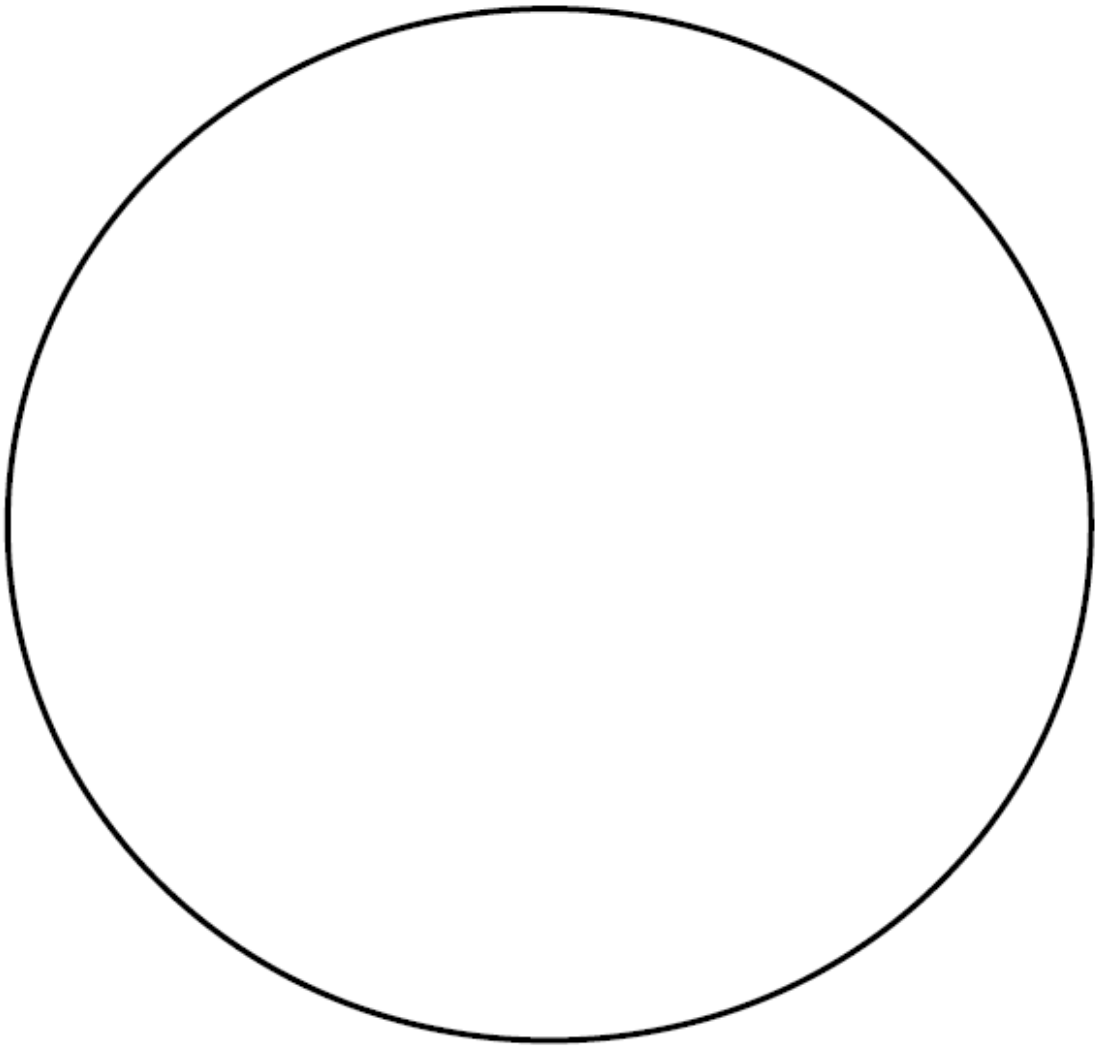
Record number of errors:

<2 errors	2-4 errors	>4 errors
normal	Mild to moderate impairment	Significant impairment with initiation or inhibition

Comment on person's ability to follow instructions / change set / speed etc.
Note any motoric impulses (e.g., hand twitching) to green and score as error.

Clock Drawing Test

Patient's Name: _____ Date: _____



Instructions for the Clock Drawing Test:

Step 1: Give patient a sheet of paper with a large (relative to the size of handwritten numbers) predrawn circle on it. Indicate the top of the page.

Step 2: Instruct patient to draw numbers in the circle to make the circle look like the face of a clock and then draw the hands of the clock to read "10 after 11."

Scoring:

Score the clock based on the following six-point scoring system:

Score	Error(s)	Examples
1	"Perfect"	No errors in the task
2	Minor visuospatial errors	a) Mildly impaired spacing of times b) Draws times outside circle c) Turns page while writing so that some numbers appear upside down d) Draws in lines (spokes) to orient spacing
3	Inaccurate representation of 10 after 11 when visuospatial organization is perfect or shows only minor deviations	a) Minute hand points to 10 b) Writes "10 after 11" c) Unable to make any denotation of time
4	Moderate visuospatial disorganization of times such that accurate denotation of 10 after 11 is impossible	a) Moderately poor spacing b) Omits numbers c) Perseveration: repeats circle or continues on past 12 to 13, 14, 15, etc. d) Right-left reversal: numbers drawn counterclockwise e) Dysgraphia: unable to write numbers accurately
5	Severe level of disorganization as described in scoring of 4	See examples for scoring of 4
6	No reasonable representation of a clock	a) No attempt at all b) No semblance of a clock at all c) Writes a word or name

(Shulman et al., 1993)

Higher scores reflect a greater number of errors and more impairment. A score of ≥3 represents a cognitive deficit, while a score of 1 or 2 is considered normal.